T-lymphocyte subsets and thymic size in malnourished infants in Egypt: a hospital-based study

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المجموعات الفرعية من اللمفاويات التائية وحجم التوتة في الرُضَّع السيئي التغذية في مصر: دراسة في مستشفى

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الخلاصة: تناولت الدراسة قياس حجم التوتة بالموجات فوق الصوتية، مع ربطها بالنسبة المئوية للمفاويات التائية من النمطين CD4 وCD8 في الدم المحيطي، لدى 32 رضيعاً مصاباً بسوء التغذية بالبروتين والطاقة، ومقارنة النتائج مع 14 من الأطفال الشواهد الأصحاء. وأوضحت الدراسة وجود ضمور التوتة في المصابين بسوء التغذية بالبروتين والطاقة، ولاسيَّما في النمط الوَذَمي منهم، مترافقاً مع تغيُّرات في المجموعات الفرعية للمفاويات المحيطية. وقد كانت هذه التغيُّرات قابلة للتراجع بعد التأهيل التغذوي، إلا أنها قد تؤثِّر على الحالة المناعية لدى الأطفال المصابين بسوء التغذية بالبروتين والطاقة، وقد تتطلَّب فترة أطول من التأهيل التغذوي تزيد عمَّا هو مطلوب لاستعادة القياسات الانثروبيولوجية. وتوصي الباحثات بإجراء تقييم مناسب للوظائف المناعية للمرضى المصابين بسوء التغذية بالبروتين والطاقة خلال فترة التأهيل التغذوي، ريثما يستعيدون عافيتهم تماماً.

ABSTRACT Thymus size was assessed ultrasonographically and correlated to the percentage of CD4 and CD8 T-lymphocytes in peripheral blood in 32 infants with protein–energy malnutrition (PEM) and compared with 14 healthy control infants. The study revealed thymus atrophy in patients with PEM, especially the oedematous type, accompanied by changes in the peripheral lymphocyte subsets. These changes were reversible after nutritional rehabilitation. However, they may affect the immune status of PEM patients and may require a longer duration of nutrition rehabilitation than required for recovery of anthropometric measures. We recommend proper assessment of the immune functions of PEM patients during nutritional rehabilitation until full recovery.

Sous-groupes de lymphocytes T et taille du thymus chez le nourrisson malnutri en Égypte : étude en milieu hospitalier

RÉSUMÉ La taille du thymus a été évaluée par échographie et corrélée au pourcentage de lymphocytes T CD4 et CD8 présents dans le sang périphérique de 32 nourrissons souffrant de malnutrition protéinocalorique (MPC), les résultats étant comparés à ceux de 14 nourrissons témoins en bonne santé. L'étude a révélé une atrophie thymique chez les patients malnutris, en particulier chez ceux présentant la forme œdémateuse de la MPC, accompagnée de modifications des sous-groupes lymphocytaires périphériques. Ces modifications se sont avérées réversibles après réadaptation nutritionnelle. Elles sont toutefois susceptibles d'influer sur le statut immunitaire des patients atteints de MPC et peuvent nécessiter une réadaptation nutritionnelle plus longue que ne l'exigerait la normalisation des valeurs anthropométriques. Nous recommandons l'évaluation minutieuse de la fonction immunitaire des patients souffrant de MPC tout au long de la phase de réadaptation nutritionnelle jusqu'à leur complet rétablissement.

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Introduction

According to Goldhagen, malnutrition is one of the leading causes of morbidity and mortality in infancy and childhood, particularly in developing countries [*1*]. While there have been some improvements in the prevalence of underweight and stunting in some regions of the world over the past 2 decades, the population of the developing world increased during this time. This means that the total number of underweight and stunted children has not changed dramatically since the early 1980s [*2*]. Thus, the scope of modern research cannot ignore the morbidity and mortality associated with the acute forms of undernutrition and malnutrition.

The function of the immune system has always been the focus of attention in malnutrition diseases in infancy. In the thymus gland, precursor cells called thymocytes develop into 2 types of immune cell: the CD4 helper T-cells which alert the immune system to an attack by a pathogen and the CD8 suppressor T-cells which destroy cells that have been damaged [*3*]. The level of T-lymphocyte subsets in peripheral blood provide information about the development and function of the immune defence system in infants [*4,5*]. A low ratio of CD4+ (helper) lymphocytes relative to CD8+ (suppressor) thymic lymphocytes is widely accepted as an indicator of the depression of thymus-dependent immune competence associated with wasting protein–energy malnutrition (PEM) [*6*].

It is the thymus gland that provides the environment for maturation of T-lymphocytes. Although the thymus size at birth may be an important predictor of immune competence [*7*], the exact significance of its size or alterations in its size in infancy in relation to the maturing immune defence system is not known [*8*]. A few previous studies have suggested that thymus atrophy is associated with severe malnutrition [*9,10*] and increased morbidity and mortality [*7*]. However, no studies have been done to demonstrate whether there is thymus atrophy in patients with PEM, both the oedematous and non-oedematous types, and whether it is a reversible condition.

This study was designed to assess the thymus size in infants with PEM and to correlate it to the peripheral blood T-lymphocyte counts, with special emphasis on the effect of nutritional rehabilitation.

Methods

Patients

The present study included 46 infants recruited from the Children's Hospital, Ain Shams University, Cairo, Egypt. There were 32 infants suffering from PEM and 14 healthy age- and sex-matched infants. All the studied infants were from low socioeconomic status families according to the classification of Park and Park [*11*].

The infants with PEM were enrolled in the study after fulfilling a set of inclusion criteria. All had dietetic errors as the cause of PEM and none had a chronic illness or any chromosomal or hereditary disorder that caused the malnutrition. All enrolled infants were breastfed in addition to receiving some traditional foods, according to their age. None of the patients was receiving any medication that would be likely to affect the immune system, whether suppressors or stimulants. The 32 malnourished children were categorized into 2 groups (non-oedematous or oedematous PEM) according to Heird's classification [*2*]. The non-oedematous group was 18 infants (8 males and 10 females) with a mean age of 12.11 [standard deviation (SD) = 4.64] months and the oedematous group was 14 infants

(5 males and 9 females) whose mean age was 12.29 [standard deviation (SD) 3.91] months.

The control children were recruited from patients presenting for dietetic advice, vaccination or circumcision (in males) at the outpatient clinic in the Children's Hospital, Ain Shams University. They were 6 males and 8 females with a mean age of 11.00 (SD 4.15) months.

Data collection

After obtaining the approval of the ethical committee of the Children's Hospital, Ain Shams University, the nature of the study was explained to the parent or legal guardian and a written consent was signed. A detailed history was taken from each child, with special emphasis on dietetic history. The mother or caregiver was asked to complete a questionnaire in simple Arabic language about how and what they fed their baby from birth until the time of admission, using a 24-hour recall of what the baby received. A thorough clinical examination was performed for all the studied infants, including anthropometric measurements (weight, height, skull circumference and mid-arm circumference), as well as routine laboratory investigations, including complete blood count (CBC), serum albumin, creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Thymus size was evaluated by ultrasonography and the CD4 and CD8 percentage in peripheral lymphocytes was estimated by flow cytometry. Both evaluations were carried out in the first 72 hours following admission depending on the patient's general condition.

All PEM patients spent a period of approximately 30 days in the paediatric ward for the initial phase of nutritional rehabilitation. All the clinical, laboratory and radiological assessments were repeated after 2–3 months of nutritional rehabilitation according to World Health Organization (WHO) recommended methods [*12*].

Nutritional rehabilitation

The WHO nutritional rehabilitation programme for the PEM infants starts with management of life-threatening and emergency conditions in the first week. Then supervised feeding starts with a calorie intake of 80–100 kcal/kg/day, keeping in mind the continuity of breastfeeding in any breastfed infants. The diet is low in protein, fat and sodium and high in carbohydrates as almost all severely malnourished infants have infections, impaired liver and intestinal functions and problems related to electrolyte imbalance. After the return of the infant's appetite the calorie intake is increased to 150–200 kcal/kg/day with an increase in the amounts and decrease in the frequency. A high-protein diet is given and vitamins and minerals (potassium, magnesium and zinc) are continued in increased amounts. Iron is given during this stage to treat anaemia. The infant remains in the hospital for the first part of this rehabilitation phase (at least 3 weeks after admission), and is then followed up in the nutritional rehabilitation outpatient clinic.

Ultrasound evaluation

Ultrasound evaluation of the thymus size was done for the cases and controls by grey-scale sonography (Logic 500, General Electric, Milwaukee, USA), with a high-resolution multi-frequency linear-array transducer, range 6–10 MHz. Ultrasonography of the normal thymus shows a homogeneous and finely granular echotexture with some echogenic strands [*13–16*]. It is located in the superior mediastinum under the sternum, anterior to the great vessels, and is easily identified in relation to the

aorta and superior vena cava [*16,17*]. Suprasternal, trans-sternal, parasternal, and intercostal approaches were used [*15,16,18*]. The thymus size was measured from the maximum diameter of the transverse axis and maximum diameter of the longitudinal axis. The measurements were repeated 3 times to ensure reliability, and average values were calculated. The longitudinal and transverse measurements were multiplied and calculated as the thymic index; this is an estimate of the volume of the thymus, and postmortem examinations have shown a high correlation between the thymic index and the weight and volume of the thymus [*19*]. Figures 1 and 2 show the ultrasound images of a PEM patient before and after nutritional rehabilitation.

Laboratory workup

For the laboratory workup, samples of blood were collected from all infants and processed as clotted venous blood and EDTA anticoagulated blood. Serum samples were used for the determination of liver and kidney functions (Synchron CX-5 Delta, Beckman Instruments, Fullerton, California, USA) and the EDTA blood was used to estimate CBC (Coulter T660, Miami, USA).

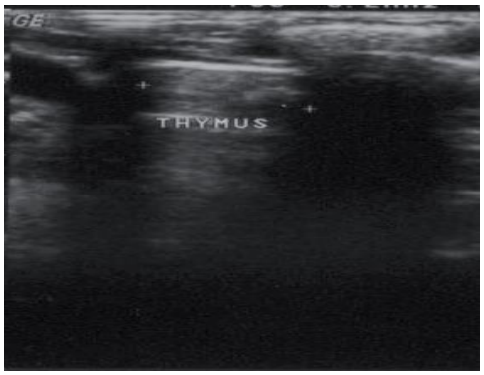

Figure 1 **Thymic size of an infant with protein–energy malnutrition before nutritional rehabilitation**

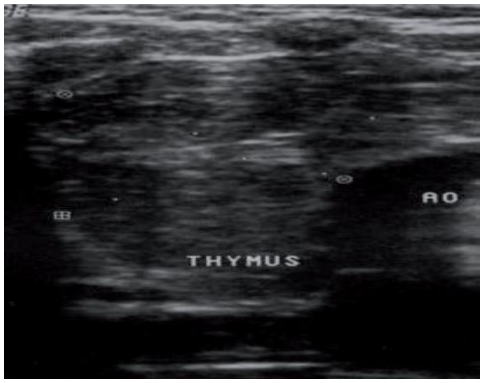

Figure 2 **Thymic size of an infant with protein–energy malnutrition after nutritional rehabilitation**

Flow cytometric analysis

The EDTA blood was used for flow cytometric assessment of the percentage of CD4 and CD8 in peripheral lymphocytes (EPICS-XL flow cytometer, Coulter, Florida, USA). The analysis was performed within 24-hours of collection using fluorescein isothiocyanate-labelled anti-CD4 and phycoerythrin-labelled anti-CD8 with their specific isotypic control reagents. All monoclonal antibodies were purchased from Becton Dickinson (Mountain View, California, USA). The collected blood was incubated with each of the 2 monoclonal antibodies for 30 minutes at room temperature. Erythrocytes were lysed by adding a lysing solution (ammonium chloride 0.85% buffered with potassium bicarbonate pH 7.2) for 5 minutes at 37 ºC. Finally, the samples were washed with phosphate–buffer saline prior to flow cytometric analysis. The lymphocytes were specifically analysed by selective gating based on the parameters of forward and side scatter. Absolute numbers were calculated from leukocyte numbers using a cell counter (T-540, Coulter,

Florida, USA) and from the proportion of lymphocytes among all leukocytes as determined by light scatter.

Statistical analysis

Statistical analysis of the results was done using *SPSS*, version 10 and *Statistica*, version 5 (Statsoft, Tulsa, Oklahoma, USA). Non-parametric data were analysed by the Shapiro Wilk's test. Student *t* and paired-*t* tests were used for parametric quantitative data and Mann–Whitney *U* and Wilcoxon matched pairs tests for non-parametric quantitative data in addition to the correlation studies. The numerical data were represented as mean (SD) and median (interquartile range). The differences were considered significant at $P < 0.05$.

Results

The study revealed lower anthropometric measurements before nutritional rehabilitation in patients with non-oedematous and oedematous PEM compared with those of the controls (Table 1). These measurements showed a significant improvement after nutritional rehabilitation, yet not reaching the control values (Table 2). The same findings were observed for serum albumin and haemoglobin levels although these values reached the control levels after rehabilitation (Tables 1 and 2). Total leucocytic count (TLC) values were significantly higher in both groups of PEM patients compared with the controls and decreased after nutritional rehabilitation (Tables 1 and 2).

When comparing the 2 types of PEM, the study also revealed a significantly lower weight (% of median for age), length/height (% of median for age) and midarm circumference, and higher serum albumin levels, in the non-oedematous compared to the oedematous patients before nutritional rehabilitation (Table 1).

The results of the CD4 and CD8 counts in oedematous and non-oedematous PEM patients, as well as the controls, before and after nutritional rehabilitation (Table 3) were within the normal values for age and sex [*20*]. However, there was higher CD4% and lower CD8% in both groups of PEM patients compared with the controls on admission (the higher CD4 was significant only in the oedematous group and the lower CD8 was significant only in the non-oedematous one). These levels almost reached the control values after nutritional rehabilitation (Table 3). As regards the CD4/CD8 ratio it was significantly higher in both groups of PEM patients compared with the controls and normalized after nutritional rehabilitation (Table 3). Non-oedematous and oedematous patients showed significant improvement in CD4/CD8 ratio after nutritional rehabilitation ($Z = 2.46$, $P < 0.01$ and $Z = 2.4$, $P < 0.05$ respectively). The thymic index showed significantly lower values in both groups of PEM compared with those of the controls and these measurements showed significant improvement after nutritional rehabilitation although not reaching the control values (Table 3). Non-oedematous and oedematous patients showed a significant improvement after nutritional rehabilitation as regards thymic index ($Z = 3.73$, $P < 0.001$ and $Z = 3.30$, $P < 0.01$ respectively).

The correlation studies revealed significant negative correlations between the thymic index before and after nutritional rehabilitation and the age of the PEM patients ($r = –0.41$ and $–0.45$, $P < 0.05$ and < 0.01 respectively). There was also a significant negative correlation between the thymic index before nutritional rehabilitation and the TLC of the PEM patients ($r = –0.37$, $P < 0.05$). In addition, there was a significant positive correlation between the thymic index before nutritional rehabilitation and the serum

Table 1 **Anthropometric measurements and laboratory parameters of infants with non-oedematous and oedematous protein–energy malnutrition before nutritional rehabilitation compared with controls**

Variable	Mean ± SD or Median (interquartile range)			Statistics		
	Non-oedematous Group 1 (*n* = 18)	Oedematous Group 2 (*n* = 14)	Control Group 3 (*n* = 14)	Group 1 vs 3 *t or Z*[a] value	Group 2 vs 3 *t or Z*[a] value	Group 1 vs 2 *t or Z*[a] value
Weight (% of median for age)	54.24 (5.98)	78.05 (9.64)	94.12 (9.44)	–4.79[a]	4.51[a]	–4.79[a]
Length/height (% of median for age)	84.42 (7.01)	88.89 (8.48)	97.14 (1.42)	*P* < 0.001 –4.79[a]	*P* < 0.001 –3.96[a]	*P* < 0.001 –3.20[a]
Skull circumference (% of median for age)	92.61 ± 2.40	93.16 ± 3.06	95.36 ± 3.33	*P* < 0.001 –2.72	*P* < 0.001 –1.82	*P* < 0.01 0.58
Mid-arm circumference (cm)	9.50 (1.13)	11.00 (2.50)	11.50 (2.00)	*P* < 0.05 –4.41[a] *P* < 0.001	*P* > 0.05 –1.31[a] *P* > 0.05	*P* > 0.05 –3.16[a] *P* < 0.01
Serum albumin (g/dL)	3.90 (0.63)	2.00 (3.00)	3.90 (0.90)	–0.31[a] *P* > 0.05	–4.53[a] *P* < 0.001	–4.82[a] *P* < 0.001
Haemoglobin (g/dL)	8.64 ± 1.01	8.81 ± 0.80	9.71 ± 0.56	–3.56	–3.23	–0.52
Total leukocytic count (/mm^3)	9.50 (3.75)	10.30 (2.70)	7.50 (1.10)	*P* < 0.01 –4.34[a] *P* < 0.001	*P* < 0.01 –4.51[a] *P* < 0.001	*P* > 0.05 –1.29[a] *P* > 0.05

[a]*Non-parametric data were detected by Shapiro–Wilk test and presented as median (interquartile range). The test of significance was Mann–Whitney test.*
SD = standard deviation.

Table 2 **Comparison between anthropometric measurements and some laboratory parameters of infants with non-oedematous and oedematous protein–energy malnutrition before and after nutritional rehabilitation**

Variable	Non-oedematous (n = 18) Median (interquartile range)			Oedematous (n = 14) Median (interquartile range)		
	Before	After	t or Z^a value	Before	After	t or Z^a value
Weight (% of median for age)	54.24 (5.98)	78.57 (13.65)	–3.73[a] $P < 0.001$	78.05 (9.64)	87.83 (8.14)	–3.30[a] $P < 0.01$
Length/height (% of median for age)	84.42 (7.01)	87.16 (8.55)	–3.73[a] $P < 0.001$	88.89 (8.48)	90.00 (8.97)	–3.30[a] $P < 0.01$
Skull circumference (% of median for age)	91.21 (3.87)	96.70 (3.42)	–3.73[a] $P < 0.001$	92.47 (3.89)	94.62 (5.73)	–3.30[a] $P < 0.01$
Mid-arm circumference (cm)	9.50 (1.13)	11.00 (2.38)	–3.75[a] $P < 0.001$	11.00 (2.50)	11.40 (2.60)	–3.32[a] $P < 0.01$
Serum albumin (g/dL)	3.90 (0.63)	4.20 (0.28)	–2.87[a] $P < 0.01$	2.00 (3.00)	3.60 (0.30)	–3.32[a] $P < 0.01$
Haemoglobin (g/dL)	8.50 (1.20)	9.90 (0.65)	–3.73[a] $P < 0.001$	9.00 (1.40)	10.00 (2.60)	–3.13[a] $P < 0.01$
Total leukocytic count (/mm^3)	9.50 (3.75)	9.50 (1.42)	–0.83[a] $P > 0.05$	10.30 (2.70)	8.30 (4.00)	–2.11[a] $P < 0.05$

[a]*Non-parametric data were detected by Shapiro–Wilk test. The test of significance was Wilcoxon matched pairs test.*

Table 3 **T-lymphocyte subsets (percentage of CD4 helper and CD8 suppressor cells) and thymic index of infants with non-oedematous and oedematous protein–energy malnutrition before and after nutritional rehabilitation compared with controls**

Variable	Mean ± SD or Median (interquartile range)			Statistics		
	Non-oedematous Group 1 (*n* = 18)	Oedematous Group 2 (*n* = 14)	Control Group 3 (*n* = 14)	Group 1 vs 3 *t or Z*[a] value	Group 2 vs 3 *t or Z*[a] value	Group 1 vs 2 *t or Z*[a] value
CD4 cell levels (%)						
Before rehab.	39.22 ± 8.98	46.45 ± 6.76	36.48 ± 3.78	1.07	–4.82	–2.51
				P > 0.05	*P* < 0.001	*P* < 0.05
After rehab.	34.20 ± 8.44	36.43 ± 7.97	36.48 ± 3.78	–0.94	– 0.20	–0.56
				P > 0.05	*P* > 0.05	*P* > 0.05
CD8 cell levels (%)						
Before rehab.	19.87 ± 6.83	23.06 ± 5.77	23.74 ± 2.03	–2.05	–0.40	–1.40
				P < 0.05	*P* > 0.05	*P* > 0.05
After rehab.	22.85 (5.25)	25.55 (6.58)	23.35 (3.18)	–0.89[a]	0.67[a]	–1.42[a]
				P > 0.05	*P* > 0.05	*P* > 0.05
CD4/CD8 ratio						
Before rehab.	2.18 (1.28)	2.06 (0.75)	1.53 (0.08)	2.81[a]	–2.67[a]	–0.46[a]
				P < 0.01	*P* < 0.01	*P* > 0.05
After rehab.	1.46 (0.25)	1.58 (0.82)	1.53 (0.08)	–1.52[a]	–0.32[a]	–0.38[a]
				P > 0.05	*P* > 0.05	*P* > 0.05
Thymic index						
Before rehab.	5.20 (5.30)	2.40 (1.50)	18.50 (5.90)	–4.79[a]	–4.51[a]	–2.98[a]
				P < 0.001	*P* < 0.001	*P* < 0.01
After rehab.	7.85 (4.60)	7.40 (2.83)	18.50 (5.90)	–4.79[a]	–4.50[a]	–0.82[a]
				P < 0.001	*P* < 0.001	*P* > 0.05

[a]*Non-parametric data were detected by Shapiro–Wilk test and presented as median (interquartile range). The test of significance was Mann–Whitney test.*

albumin of the PEM patients ($r = 0.45$, $P = 0.01$).

No correlation between the size of the thymus and CD4%, CD8%, or the CD4/CD8 ratio could be detected in the controls. However, the current study revealed negative correlations between the thymic index before nutritional rehabilitation and CD4% and CD8% ($r = –0.31$ and $–0.42$, $P > 0.05$ and < 0.01 respectively) and a positive, though non-significant, correlation with the CD4/CD8 ratio ($r = 0.06$, $P > 0.05$).

Discussion

The current study revealed higher CD4% and lower CD8% and subsequently higher CD4/CD8 ratio in both groups of PEM patients compared with the controls on admission (the higher CD4 was significant only in the oedematous group and the lower CD8 was significant only in the non-oedematous one). These levels almost reached the control values after nutritional rehabilitation.

Contrary to the results of the current study, Freitag et al. found a decrease in CD4 percentage and CD4/CD8 ratio and increase in CD8% in an animal model of starvation [*21*]. During the refeeding period, increases were observed in the CD4%, the CD8% and lymphocyte number.

In agreement with the results of the current study, the higher CD4% was encountered earlier in kwashiorkor and marasmic kwashiorkor patients (oedematous PEM) by Rikimaru et al., yet the authors did not comment on this finding [*22*]. However, the same study reported higher CD8% in the same patients; thus the CD4/CD8 ratio was lower in them compared to the controls. In addition, Najera et al. reported that CD8% percentage in malnourished infected children was non-significantly lower than the well nourished non-infected controls [*23*], which is in agreement with the present study.

The increased CD4% could be a compensation for the lower proportion of B-cells which is needed to fight infections in PEM patients. Rikimaru et al. reported that the proportion of B-cells was significantly lower in severely malnourished children than in normal children [*22*]. More recently, Najera et al. explained that there is an inability to increase the proportion of B-lymphocytes in malnutrition which may be associated with the mechanisms involved in the immunodeficiency of malnourished children [*23*].

The results of the present study could also be explained by the work of Woodward and Miller [*24*]. They reported that in weanling mice the low-protein diet protocol exerted no influence on the CD4/CD8 T-cells ratio, which challenges the established concept that T-dependent immunodepression in PEM depends on a reduced CD4/CD8 ratio. They also added that the low-protein diet protocol increased the ratio of T-cells to B-cells in the secondary lymphoid organs and recirculating pool; thus the fact that PEM induces greater involution within the T-cell system than within the B-cell system was also challenged. Moreover, Lee and Woodward reported that the CD4/CD8 ratio is irrelevant to the thymus-dependent immune incompetence that they demonstrated in their rodent models [*6*].

The present study demonstrated a significantly lower thymic index in both groups of PEM patients compared with those of the controls. Nezelof [*9*] and McMurray [*10*] previously reported that severe thymus atrophy is secondary to various causes, including prolonged protein malnutrition.

The thymic index of the currently studied PEM patients showed significant improvement after nutritional rehabilitation. This is

consistent with Savino, who reported that the thymus atrophy present in malnutrition can be reversed after appropriate diet rehabilitation [*25*]. In spite of such significant improvements, the studied PEM patients still showed significantly lower thymic index values compared with the controls. This could be explained by the study of Chevalier et al. who reported that immune recovery of malnourished children takes longer than nutritional recovery [*26*].

A significant negative correlation was found between the thymic index before and after nutritional rehabilitation and the age of the PEM patients, which is similar to the report of Hasselbalch et al. on healthy children aged 8 to 12 months [*27*]. There was also a significant negative correlation between the thymic index before nutritional rehabilitation and the TLC of the PEM patients, denoting that morbidity (infection) is greater in PEM patients with low thymic index. This is in agreement with Aaby et al. who reported that thymus atrophy is associated with severe malnutrition and increased morbidity and that larger thymus size was associated with lower infant mortality [*7*].

The results of the present study also agree with Hasselbalch et al. [*27*], who concluded that most of the individual variation in thymus size in infants can be explained primarily by body size and to a lesser extent by illness. They found a greater significant correlation between body size and thymic index than with the previous history of fever episodes and thymic index. The present study similarly revealed a highly significant lowered thymic index values in PEM patients, who have significantly lower anthropometric measurements, compared with those of the controls, while the negative correlation between TLC and thymic index was hardly significant.

The present study revealed that oedematous PEM patients had a significantly lower thymic index than non-oedematous ones. In addition, there was a significant positive correlation between the thymic index before nutritional rehabilitation and the serum albumin of the PEM patients. These results can be explained by the fact that oedematous PEM is the more severe form of malnutrition, with lower serum albumin and zinc levels [*28*], and even the oral zinc tolerance test was found to be more affected in such patients [*29*]. Savino reported that malnutrition that is secondary to deficiency in uptake of proteins, metal elements or vitamins consistently results in changes in the thymus gland [*25*]. Moreover, McMurray previously specified that PEM and zinc deficiency are major causes of thymic atrophy [*10*].

Similar to our results for the controls Hasselbalch et al. could not find any correlation between the size of the thymus and the CD4% or CD8%, or the CD4/CD8 ratio in healthy newborn infants [*30*]. However, the present study revealed negative correlations in PEM patients between thymic index before nutritional rehabilitation and CD4% and CD8%, yet only the latter was significant and positive, though non-significant, correlation with the CD4/CD8 ratio. Jeppesen et al. reported that it is the decreased number of immature lymphocytes from the thymic cortex and not the mature T-lymphocytes that could be correlated to the thymic size [*8*]. Additionally there are many other factors affecting these T-lymphocyte subsets counts, for example, cytokines [*31–33*] and infections [*34–36*].

In conclusion, the current study revealed thymic atrophy in PEM patients, especially infants suffering the oedematous type, accompanied by changes in the peripheral lymphocyte subsets. These changes are likely to affect the immune status of PEM patients and could be detrimental in this young age. Fortunately they are reversible

upon nutritional rehabilitation, although they might need a longer duration than physical recovery. We thus recommend proper assessment of the immune functions of PEM patients during nutritional rehabilitation and thereafter until full recovery.

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