Role of thymic ultrasonography in the diagnostic workup of infants with protein–energy malnutrition

Mona El-Kalioubie, Wessam Abd El-Rahman El-Zayat, Mariam Raafat

Diagnostic and Intervention Radiology Department, Cairo University Hospitals, Kasr Al-Ainy, Cairo, Egypt

Correspondence to Mona El-Kalioubie, Diagnostic and Intervention Radiology Department, Cairo University Hospitals, Kasr Al-Ainy, El-Manial, 11956 Cairo, Egypt; Tel: +20 109 667 5384; e-mail: monaelkalioubie@icloud.com

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Purpose
The aim of this study was to report the significance of quantitative thymic gland analysis using ultrasonography (US) in the diagnostic workup of infants with protein–energy malnutrition (PEM) to assess thymic index and correlate it with anthropometric and peripheral blood lymphocyte subset measurements.

Patients and methods
US assessment of thymic size was carried out prospectively in 36 infants with PEM, compared with 16 control infants. Correlation with anthropometric measurements and peripheral total leukocytic count as well as peripheral CD4/CD8 T-lymphocytes ratio was made.

Results
Significant thymic atrophy and reduced anthropometric measurements were found in all patients. Leukopenia and significantly lower CD4/CD8 ratio were also found. A positive weak correlation was noted between the thymic index and the CD4/CD8 ratio in PEM infants (r=0.4695; P=0.004). A weak negative correlation was found between the thymic index and the CD4/CD8 ratio in controls (r=0.0813; P=0.76).

Conclusion
An easy, rapid, and especially noninvasive method such as US provides a suitable quantitative method to assess the thymus gland. In infants with PEM, thymic atrophy is associated with changes in the peripheral lymphocyte subsets with detrimental sequelae on the immune system, justifying the use of US for indirect evaluation of the immunocompetence level in malnourished children.

Keywords:
immunosupression, infants, malnutrition, thymus, ultrasonography

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Introduction

Malnutrition during infancy is regarded as the most relevant risk factor for illness and death and is still a major burden in developing countries [1]. Protein–energy malnutrition (PEM) is defined by measurements falling more than two standard deviations (SVs) below the normal weight and height relative to age (underweight and stunting, respectively) and below normal weight for height (wasting) [2]. With poverty at the top of the list, many other factors can affect the degree and distribution of PEM, such as socioeconomic and political instability, poor sanitary conditions, poor diet practices and knowledge, improper education, breast feeding habits, and ineffectiveness or shortage of nutrition rehabilitation programs [1,3].

Literature reports have long stated that the thymus gland, which provides the environment for T-cell differentiation in small children, undergoes several alterations in seriously malnourished infants, associated with a severe involution of the organ [4,5]. In particular, in severe forms of PEM, the term nutritional thymectomy has been used, and related cellular immunity depression has been described with disturbances in the T-lymphocyte subpopulations and an increased number of immature T-cells [6]. This picture has been described as reversible after adequate nutritional rehabilitation, taking, however, longer than the recovery of anthropometric measurements [7].

Previous studies have shown that ultrasonography (US) may be used for correct imaging of the thymus in infants. Unlike computed tomography that involves contrast medium and sedative injection and shows a disproportionate cost to the income of developing countries, real-time US does not show such inconveniences, and sonographic reports of the thymus appearance and size have been published in both healthy and malnourished infants [8,9].

The aim of this study was to present the methodological basis and report the significance of quantitative thymic gland analysis using US in the diagnostic workup of infants with PEM, to assess thymic index, and correlate it with anthropometric measurements and changes in the peripheral blood lymphocyte subsets.

Patients and methods

Study population

After parental consent, infants were selected from the outpatient clinic at the Children's Hospital, Cairo University, Egypt, between May 2015 and August 2016, with the approval of the hospital's ethical committee.

A total of 36 (14 male and 22 female) infants of both sexes with an initial diagnosis of PEM were recruited, with a mean±SD age of 7.4±2.8 months. All patients originated from poor homes in suburban Cairo and nearby governorates. Low-income families, lack of sanitation, poor diet, and early weaning were characteristics. However, four patients had PEM secondary to chronic renal disease.

PEM diagnosis was based on the use of a cutoff for weight-for-height of below −3 SD of the WHO standards, or mid-upper arm circumference less than 115 mm, and clinical findings such bilateral pitting edema, loss of subcutaneous tissue, and decreased muscle mass [10,11].

None of the recruited infants was receiving immune system-altering medications.

The patients were compared with 16 (10 male and six female) well-nourished healthy infants from the outpatient department (mean±SD age: 6.3±2.8 years old).

Methods

Ultrasonography imaging

The thymus gland was assessed in the entire study population using mediastinal gray-scale real-time US (LOGIQ_7 PRO; GE Medical System; kretztechnik AG, Zipf, Austria) using a high-resolution linear-array 7-MHz transducer.

Anatomically, the thymus gland is situated in the superior–anterior mediastinum, in front of the large vessels [7,8]. At US imaging, it can be recognized as a well-defined echo-poor structure, finely granular with some echogenic strands in the anterior mediastinum [7]. Scanning was performed in the supine position with arms held alongside the body.

Transternal and left parasternal approaches (as the left thymic lobe is usually more developed) were used to obtain the largest transverse and longitudinal diameters of the gland, which were subsequently multiplied and calculated as the thymic index, which is a sonographic estimate of the thymic volume [12].

Collection of clinical and laboratory data

The following data were collected from both patients and controls, from the outpatient clinical records with the help of the appointed pediatric residents:

(1) Anthropometric measurements including weight, height, mid-arm, and skull circumference.
(2) Blood pictures were analyzed for total leukocytic counts (TLCs). CD4/CD8 ratio (in peripheral lymphocytes) obtained using flow cytometry in mononuclear cell suspensions from peripheral blood was also reported.

Statistical analysis

The statistical package for the social sciences software (SPSS; SPSS Inc., Chicago, Illinois, USA), release 21 versions for Mac was used. Data were expressed as mean±SD or number (percentage). The quantitative parametric data were compared between patients and controls using the unpaired Student *t*-test.

Pearson's correlation studies were carried out between the thymic index and laboratory data.

The *P* values using the χ^2-test were determined: *P* less than 0.05 was considered significant, *P* less than 0.01 was considered highly significant, and *P* more than 0.05 was considered nonsignificant.

Results

Ultrasonography findings

The thymus gland in the malnourished infants showed severe involution, as detected by a significantly lower thymic index compared with the control group (Fig. 1): mean thymic index 5.5±2.3 versus 16.8±6.5 (patients vs. controls) ($P<0.0001$) (Figs 2 and 3).

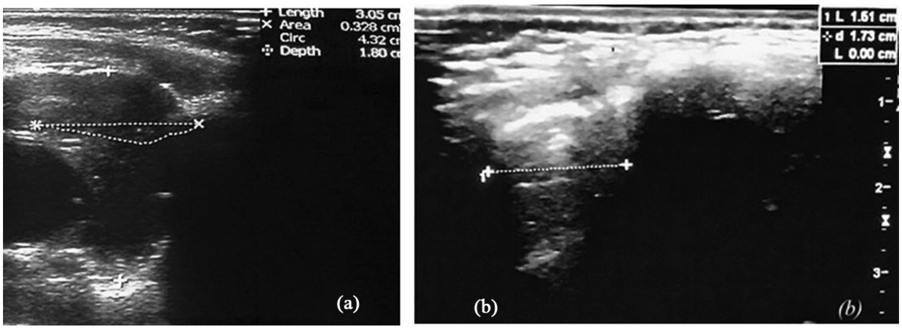
Anthropometric findings

Significantly lower anthropometric measurements were reported in patients with PEM when compared with the healthy controls ($P<0.0001$) (Table 1).

Peripheral blood lymphocyte findings

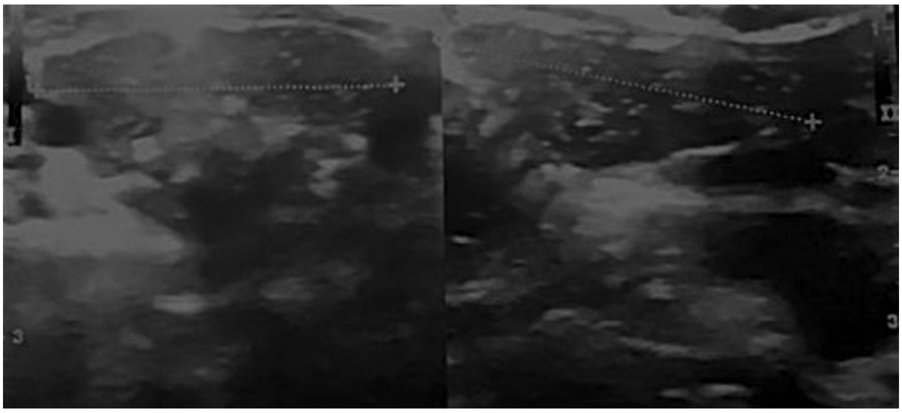
Leukopenia with decreased TLC was found in PEM patients when compared with the controls: mean TLC 5.1±1.1 versus 7.6±1.3 mm^3 (patients vs. controls) ($P<0.0001$).

Figure 1



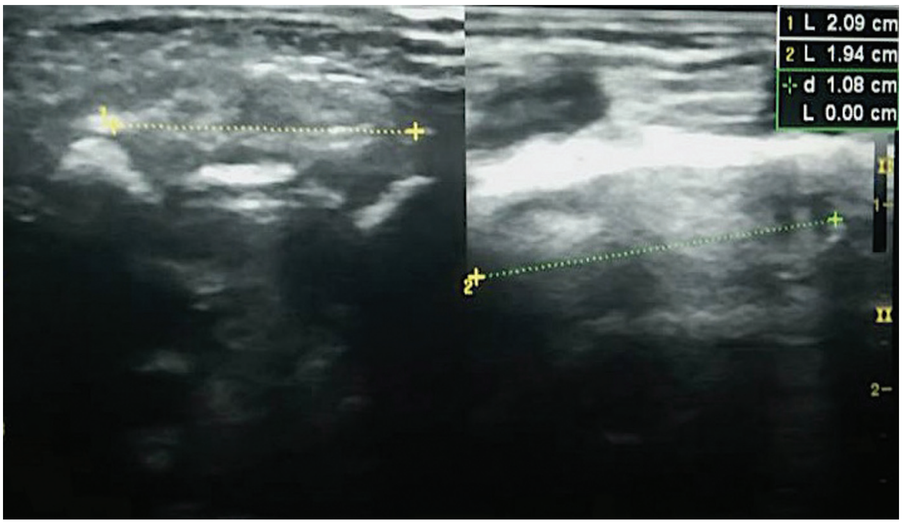
(a) Transverse ultrasound image of the thymus in a control infant (6 months). (b) Transverse ultrasound image of the thymus in an infant (7 months, weight: 4.4 kg, height: 59 cm) with protein–energy malnutrition, showing markedly hypoplastic gland.

Figure 2



Transverse and longitudinal ultrasound images of the thymus in an infant (6 months, weight: 4.2 kg, height: 62 cm) with protein–energy malnutrition, showing low thymic index (6.4).

Figure 3



Transverse and longitudinal ultrasound images of the thymus in an infant (8 months, weight: 5.2 g, height: 66 cm) with severe protein–energy malnutrition, showing low thymic index (4.05).

Flow cytometric analysis revealed a significantly lower ratio of cellular numbers within CD4 relative to CD8 in PEM patients compared with the controls: mean CD4/CD8 ratio 0.98±1.1 versus 1.7±0.8 (patients vs. controls) (P=0.0227).

Table 1 Anthropometric measurements of infants with protein–energy malnutrition compared with controls

Measurements	Protein–energy malnutrition patients (mean±SD)	Controls (mean±SD)
Weight (% of mean for age)	45.2±4.7	89.1±9.3
Height (% of mean for age)	86.8±8.1	98.3±1.3
Mid-arm circumference (cm)	8.9±1.1	11.8±1.9
Skull circumference (% of mean for age)	89.9±2.1	94.2±2.9

However, it is worth noting that four patients with PEM had higher CD4/CD8 ratio values compared with the average ratio value in healthy infants.

Nevertheless, CD4 and CD8 counts were within normal range for age and sex [13] in both studied groups.

Correlative studies

There was technically a positive correlation between the thymic index and the CD4/CD8 ratio in PEM infants (*r*=0.4695); however, the relationship between the variables is weak, but still statistically significant (*P*=0.004) (Fig. 4).

A weak negative correlation was found between the thymic index and the CD4/CD8 ratio in controls (*r*=0.0813, not statistically significant, *P*=0.76) (Fig. 5).

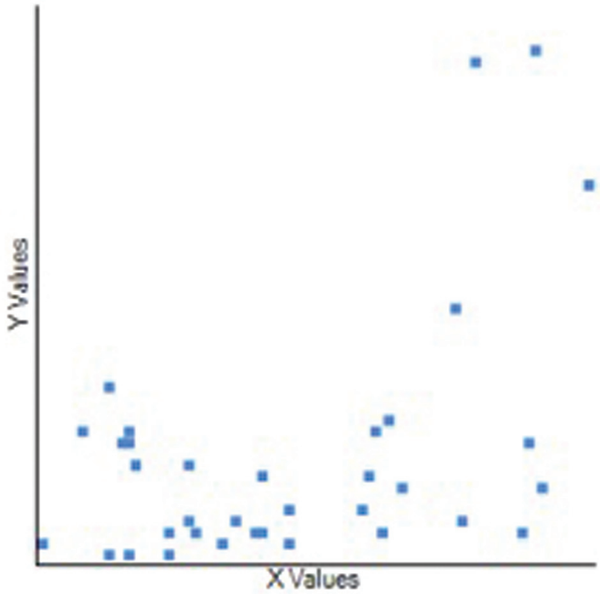
Discussion

In this study, we assessed the role of US as an easy way to determine the thymic size in infants with PEM. Knowledge of the normal range of thymic size is needed to properly diagnose pathological diseases affecting the size of the gland. The thymus involutes with age but also with excessive malnutrition and severe infections [14,15].

Long before the discovery of the immunological role of the thymus gland by Miller [16], atrophy of the gland in malnourished patients has been reported by Beisel [17] Aschkenasy [18].

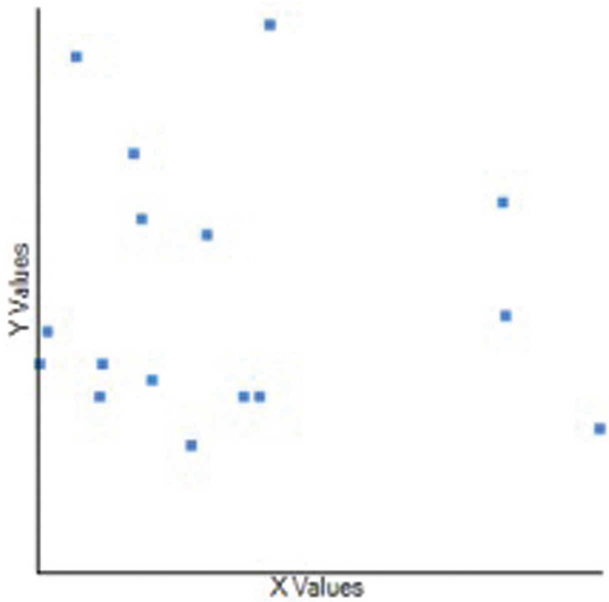
The current study showed a significantly lower thymic index in PEM infants, compared with the control group along with related significantly reduced anthropometric measurements. Chevalier reported similar results in a group of 42 children of both sexes who were hospitalized for severe PEM. Compared with the control group, the standardized area of the left thymic lobe was about 1/10th in comparison with controls [2]. Nassar *et al.* [7] also reported significantly reduced thymic index values in a group of 32 infants with PEM (both edematous and nonedematous types) that increased following nutritional rehabilitation, although not reaching the values obtained in the control group. Similar findings were reported by Rytter *et al.* [19], in a study conducted on 120 children, 53 of whom presented with

Figure 4



Positive weak Pearson's correlation between the thymic index and the CD4/CD8 ratio in PEM infants (*r*=0.4695).

Figure 5



Weak negative Pearson's correlation between the thymic index and the CD4/CD8 ratio in controls (*r*=0.0813).

edematous malnutrition. Thymus area was used as a marker, and a positive association was found between the size of the thymus gland and anthropometric indices of nutritional condition.

Savino [4] reported the targeting of the thymus gland in PEM, as related to an apoptosis-induced thymocyte depletion, particularly involving the immature CD4 and CD8 cells, as well as a decrease in cell proliferation. The thymic microenvironment was also described as affected by morphological changes in thymic epithelial cells, as well as a decrease in their thymic hormone production [4].

The present study revealed a decreased TLC as well as a lower CD4/CD8 ratio in PEM patients compared with the control group.

Rytter *et al.* [20] reported higher CD8 percentage in kwashiorkor and marasmic kwashiorkor patients (edematous PEM); thus, the CD4/CD8 ratio was lower in them compared with controls. The immediate consequence of thymic atrophy has been described as leading to leukopenia, reduced CD4/Cd8 ratio, and increased number of immature T cells in the periphery [2]. These disturbances in the thymus have been more intensely investigated in malnutrition experimental models. This is similar to the results of Freitag *et al.* [21] in which a decrease in CD4 percentage and CD4/CD8 ratio and an increase in CD8 percentage in an animal model of starvation were described. This has been, at least partially, attributed to lower leptin levels during starvation or malnutrition [4,22].

In contrast, Woodward and Miller [23] reported that, in weanling mice, the low protein-diet protocol exerted no influence on the CD4, CD8 T cells ratio, questioning the established concept that T-dependent immunosuppression in PEM depends on a reduced CD4/CD8 ratio. Similar results were stated by Nassar *et al.* [7] who reported higher CD4 percentage and lower CD8 percentage and subsequently higher ratio in PEM patients.

In the current study, it is worth noting that four patients with PEM had higher CD4/CD8 ratio values than the average ratio value in healthy infants.

Nájera *et al.* [24] also reported that CD8 percentage in malnourished infected children was not significantly lower than that in the well-nourished noninfected controls.

There was technically a positive weak correlation between the thymic index and the CD4/CD8 ratio in PEM infants (r=0.4695), in the present study.

Nassar *et al.* [7] found a negative correlation in PEM patients between thymic index before nutritional rehabilitation and CD4% and CD8%, and a positive, although nonsignificant, correlation with the CD4/CD8 ratio. A negative correlation between the thymic area and the level of immature lymphocytes was described by Chevalier [6].

A weak negative correlation was found between the thymic index and the CD4/CD8 ratio in controls (r=0.0813). Hasselbalch *et al.* [14] 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.

Jeppesen *et al.* [25] 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. In addition, there are many other factors affecting these T-lymphocyte subsets counts – for example, cytokines [26–28] and infections [29,30].

A limitation of this study was the unavailability of follow-up of the patients after nutritional rehabilitation. During the treatment of severely malnourished children, the time needed for immune recovery, based on the US assessment of the area of the left thymic lobe and the percentages of T lymphocyte subpopulations, was much longer than the time for nutritional recovery, based on anthropometry [9]. The lag between immune and nutritional recoveries implies a follow-up of the immunological status and a control diagnosis before discharge, to prevent relapses of recovered children who are nutritionally healthy but immune-depressed.

Conclusion

The current study agrees with literature reports of thymic atrophy associated changes in the peripheral lymphocytes subsets with detrimental sequelae on the immune system of PEM infants. These findings justify the use of US of the thymus gland for indirect evaluation of the immunocompetence level in malnourished children. An easy, rapid, and especially noninvasive method *in situ*, such as US, usable in children from birth in the same sonic window, confirmed the relevance of using this technique to assess the thymus.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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