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THYMIC ULTRASONOGRAPHY in CHILDREN, A NON-INVASIVE ASSESSMENT OF NUTRITIONAL IMMUNE DEFICIENCY

Chevalier P, PhD[1]. Laboratoire Nutrition Tropicale, ORSTOM, Montpellier, France.

ABSTRACT

The depression of cellular immunity in children suffering from severe protein-energy malnutrition was characterized by a decreased number of peripheral T lymphocyte subpopulations and an increased number of immature T lymphocytes. As previously studied, the T lymphocyte immaturity is linked to a deficit of thymulin from the thymic epithelium and to a critical atrophy of the thymus. Hence, the thymus might be considered as the first target for immuno-nutritional studies. During the treatment of severely malnourished children, nutritional recovery, based on anthropometric criteria such as weight for height, was reached in one month. Immunological recovery, based on percentages of the T lymphocyte subpopulations and thymic area, required two months. Because of the lag between nutritional and immunological recoveries, a discharge based only on anthropometrical parameters generated immune-depressed children. The great variation between thymic atrophy on admission and thymic recovery on discharge suggested that the initial diagnosis and the follow-up of the immunological status of malnourished children can be indirectly assessed *in situ* by a non-invasive method such as ultrasonography of the thymus.

KEY WORDS: Protein-Energy Malnutrition, Children, Thymus, Ultrasonography

INTRODUCTION

In a recent data-analysis from 53 developing countries, Pelletier et al. [1] found that more than half of child deaths are due to the malnutrition-infection synergism; the majority died of mild-to-moderate forms of malnutrition (defined as 60-79% of median weight-for-age) [2]. However, immune assessment has not commonly been investigated in malnourished children, who are particularly sensitive to opportunistic infections.

Severe protein-energy malnutrition (defined as below the reference point of 60 % of median weight-for-age) [2] produces an extreme involution of the thymus, sometimes termed nutritional thymectomy [3], a depression of cell-mediated immunity with disturbances in subpopulations of T lymphocytes, and a high level of null-cells or immature T-cells [4]. A study on Senegalese children who died of severe malnutrition showed a depleted thymulin content of the atrophied thymus [5] and is in accordance with previous observations by Chandra [6]. A 2-hour incubation

[1] ORSTOM, Laboratoire Nutrition Tropicale, BP.5045, 34032 MONTPELLIER Cedex 1, France

with thymulin halves the high level of immature T-cells [7] and confirms that the high level of immature T-cells corresponds to a defect of the lymphocyte-differentiating function of the thymic epithelium [5].

The atrophy of the thymus during malnutrition was reported a century and half before the discovery of its immunological function [8 ,9]. Hence, the thymus, « the barometer of nutrition » [10] and « the key-organ of immunity » [11], might be considered as the first target for immuno-nutritional studies. For a long time, necropsy was the only means of studying the thymus. Earlier studies in situ done more than 20 years ago, used X-ray radiography [12], but the unreliability of the technique and danger from radiation lessened this procedure to explore the thymus gland. Among the new imaging technology, the computed tomography includes contrast medium and sedative injection [13] and its cost is disproportionate to the disposable income of developing countries. The real-time ultrasonography does not present those inconveniences and was used by our team for 12 years in nutritional studies in several developing countries.

The aim of this paper is to give the methodological bases of thymic ultrasonography in preschool children and report its significance for nutritional rehabilitation of malnourished children.

SUBJECTS AND METHODS

After parental consent, children were selected, from those hospitalized in the Materno-Infantil « German Urquidi » Hospital in Cochabamba (Bolivia). A 3-stage protocol was approved by the ethical committee of the hospital.

The first stage compared 42 children of both sexes who were hospitalized for severe protein-energy malnutrition (PEM) to 15 well-nourished children of both sexes who were recruited from healthy children from the outpatient department [7]. Severe forms of PEM : Kwashiokor, Marasmus and combined forms, were based on weight-for-height (below the 80% of the NCHS reference), arm per head circumference ratio and clinical findings such as: presence of edema, loss of subcutaneous tissue and diminished muscle mass.

The second stage was a 2 month follow-up study of 45 severely malnourished children of both sexes, admitted to the CRIN (Centro de Rehabilitación Inmuno-Nutritional) [14].

The third stage was a case-history cohort study and consisted of 32 severely malnourished children of both sexes, admitted for a 2-month period to the CRIN, receiving upon admission 2 mg of zinc element per kg per day. Each of the selected children was matched, according to the nutritional pathology, age, sex and anthropometrical criteria, with one child receiving the same treatment pattern without zinc supplement [15].

Thymus size was assessed weekly by mediastinal real-time ultrasonography using an echo-camera (ALOKA SSD-210 DXII, Tokyo) with a 5 Mhz linear pediatric probe. Children were scanned in the supine position, with their arms held alongside the body. The extension of the back was obtained by placing a pillow or a cushion under the shoulders.

Because the ossification of the sternum is incomplete and the left lobe of the thymus generally more developed, we chose to scan the left side of the thorax using a parasternal approach by exploring directly over the costicartilage in a longitudinal plane. The transducer was placed perpendicular to the sternum, one inch to the left of the midline between the suprasternal notch and the xiphoid. The thymus presented a posterior molding with adjacent structures: heart and great vessels, whose mobility hampered the moment chosen to freeze. In order to assess the thymus mass, we determined a two-dimensional measure of the echographic image. To shorten the time of examination, we duplicated the echographic image on the screen. The area of the left thymic lobe between 2nd and 4th ribs was calculated with a planimeter.

RESULTS

Three studies have been carried out. In the first, the thymus gland of the malnourished children presented a severe involution. Compared to the control group, the standardized area of the left thymic lobe (ALT) for the malnourished group was on an average of about 1/10th of the control group (table 1).

In the second study, the kinetics of thymic recovery showed a 5 fold and 9 fold increase after 5 and 9 weeks, respectively, of nutritional rehabilitation. After 9 weeks, the ALT reached a value of 350 mm², which may be considered the threshold of normality established from previous studies [16].

TABLE 1

Evolution of the thymic area during hospitalization of malnourished children.

Studies	Groups	N	Thymic area (mm²)
Transversal	M	42	48.1 ± 4.7
	C	15	446.3 ± 19.3 ***
Follow up	MA	44	38.9 ± 3.2
	M1	38	193.4 ± 18.5 ***
	M2	37	348.7 ± 21.1 ***
Zinc control	CMA	32	70.6 ± 10.0
	CM1	26	229.8 ± 22.0 ***
	CM2	23	387.8 ± 25.0 ***
Zinc supplement	ZMA	32	81.3 ± 7.4
	ZM1	26	362.2 ± 26.0 ***
	ZM2	23	453.0 ± 17.3 **

Mean ± SEM; significance with previous row : ** p< 0.01; *** p< 0.001. M: malnourished children, C : controls. MA, M1, M2: malnourished children upon admission, after 1 month, after 2 months. CMA, CM1, CM2:, malnourished children without zinc supplementation (controls). ZMA, ZM1,ZM2: malnourished children with zinc supplementation

In the third study, the group with zinc supplement reached the threshold of 350 mm² after only one month and registered a similar value to the first control group in 2 months. Zinc induced a 4.5-fold increase from admission value while the same diet without zinc supplement induced a 3-fold increase for the same settled time.

Figure 1 illustrates the differences between the thymic area of malnourished and control children and the changes of the thymic area from admission to discharge during rehabilitation of the malnourished children.

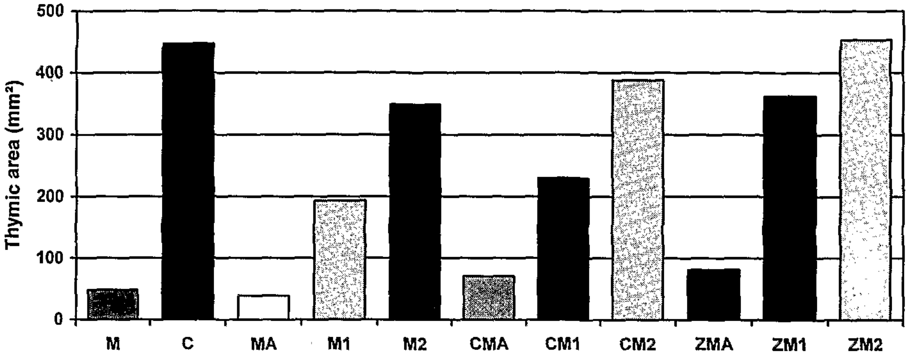

FIG. 1. Evolution of thymic area in malnourished children M: malnourished children, C : controls. MA, M1, M2: malnourished children upon admission, after 1 month, after 2 months. CMA, CM1, CM2:, malnourished children without zinc supplementation (controls). ZMA, ZM1,ZM2: malnourished children with zinc supplementation.

DISCUSSION

Compared to the first qualitative estimation of the thymus size using X-rays performed 20 years ago by Golden et al. [17], ultrasonography provided a suitable quantitative method to assess the thymus gland.

Cardiac movements during the real-time ultrasonography of the thymus modified the size of the gland and induced a high variability of the thymic values. Despite this, the difference between the thymic area of malnourished and control infants (1 to 9) confirmed the involution of the thymus gland [7]. Likewise, the range of variation between the thymic area of malnourished children upon admission and upon discharge (500 to 900%) was sufficient to evaluate the recovery of the thymus [14].

During the treatment of severely malnourished children, the time needed for immune-recovery, based on the ultrasonographic 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 [14]. 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.

The negative correlation between the thymic area and the level of immature lymphocytes [14] justified the use of the thymus to evaluate the level of nutritional immune deficiency in malnourished children. An easy, rapid and especially non-invasive method *in situ* such as ultrasonography, usable in children from birth to 6 years old in the same sonic window, confirmed the relevance of using this technique to assess the thymus.

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