A Preliminary Study: The Immune Status of Some Malnourished Filipino Preschool Children

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A variety of anthropometric indices are currently being used to assess growth status of children, particularly the preschool children. In using these indices, the growth retardation are expressed in terms of different degrees or levels of undernutrition. The functional meaning of these levels of nutritional status lies on the relationship between physical size on one hand, and functional consequences on the other. The latter refers to the influence on any of the following: work performance, physical and mental development, resistance to infection, pregnancy, lactation and fertility. The functional consequence of interest in this study, is in the area of resistance to infection using immunological parameters.

The importance of undernutrition as a public health problem lies ultimately on its consequence on health. Infectious disease morbidity is observed not only in the severely malnourished but also in the mild and moderately undernourished children. This phenomenon suggests a probable impairment of the host defense system. However, the extent and severity of the immune dysfunction at the various levels of nutritional status are yet to be fully illucidated.

The effects of severe malnutrition on the different components of the host defense system have been widely investigated. Previous studies have shown that the cell-mediated immune response is the component of the host defense system which was consistently impaired in severe malnutrition. On the other hand, studies on the immune dysfunction in mild and moderately undernutrition have been addressed by only a few workers (Reddy et al. 1976: McMurray et al. 1981; Neumann et al. 1975; Chandra 1982). Various aspects of the immune dysfunction observed in mild and moderate undernutrition still require further study, such as: a) the association and correlation of various parameters of the host defense system with the level of undernutrition classified by each anthropometric indicator (whether weight for age, weight for height or height for age) b) the comparison of the extent and severity of the immune dysfunction in stunting and in wasting. The need to re-examine the problem from the point of view of the above stated perspective is thus emphasized.

In the Philippines, undernutrition accompanied by an alarmingly increasing rate of morbidity has become a major health problem in the past years. The morbidity situation is typical in less developed countries where the leading causes of illness are still the communicable and infectious diseases. Influenza, diarrhea/gastroenteritis, bronchitis, emphysema, tuberculosis and whooping cough have remained the leading causes of morbidity through the years. It must be noted that most of them are nutrition-related diseases.

There is little published work on the immune status of Filipinos (Agbayani and Caviles, 1974) and none on the immune status of undernourished preschool children. There is an urgent need to know the seriousness of the implication of the malnutrition problem in our Filipino children by way of determining the corresponding immunological status to provide an insight on the vulnerability of the children to infectious diseases. Considering the widespread undernutrition problem and morbidity reports in the country, it is importnt to know the extent and severity of the immune dysfunction in the undernourished preschool Filipino child population as well as to know the immune status of the well-nourished ones. This information could provide some data on the immune status of our preschool children as well as know how vulnerable and susceptible to infectious diseases the mild and moderately undernourished children are, by virtue of their immune status. This study would like to provide such initial information. Specifically, this study would like to determine the immunological status of some well-nourished and undernourished preschool children by measuring some parameters of the humoral and cell-mediated immune response.

Key words: immune status, malnutrition immunity, and malnutrition.

MATERIALS AND METHODS

Sampling of the Subject

About 137 preschool children, age ranging from 1-6 years, who have had the BCG immunization were enrolled in this study through the health centers in Parks Ricarter and Dagohoy, UP Diliman. The mothers were asked to sign a contract of consent for their children to participate in this study. The age and sex distribution of the subjects are shown in Table 1.

Routine physical examination were done on all the subjects by a physician. The age, sex, height and weight of the subjects were obtained for the assessment of the nutritional status. Blood samples were extracted for the immunological laboratory tests. All these activities together with the tonsillar size measurement and the intradermal application of the antigen PPD for the skin test were done on the same sampling day so as to document immunological status at the time of the nutritional status assessment.

TABLE 1. Age and Sex Distribution of Filipino Preschool Subjects

AGE	Male	Female	Total
6 - 12 months	6	7	13
13 - 24	9	13	22
25 - 36	15	17	32
37 - 48	12	11	23
49 - 60	11	9	20
61 - 72	10	11	21
73 - 78	3	3	6
Total	66	71	137

Assessment of Nutritional Status

The age, sex and anthropometric measurements (height and weight) were obtained on the scheduled sampling day. The weight of the subjects were taken using a Salter Scale Balance.

The height or length of the subjects under two years old was measured horizontally using a length board of the infantometer. For the older subjects, a stadiometer or an anthropometric rod was used.

In the assessment of nutritional status, the anthropometric measurements taken were compared to reference or standard national values and were expressed as Percent Reference Values. The formula for the calculation of the percent reference value is given below.

$$\text{Percent Reference Value} = \frac{\text{Subject's measurement}}{\text{Reference Value}} \times 100$$

For the above formula, the recommended weight for age, weight for height, and height for age standard values for 0 to 6 years old Filipino children, set by the FNRI-DOST (1983) were used as reference values. Using the percent weight for height values and the percent height for age values the subjects were grouped according to Waterlow's classification.

Immunological Parameters' Determination

The following functional parameters of both the humoral and cell-mediated immune response were determined.

a) Tonsil size measurements using the criteria set by Smythe et al (1971)

b) Total peripheral lymphocyte count

c) Percent Rosetting T-lymphocytes

d) Delayed hypersensitivity skin test using PPD

e) Serum IgG and IgM level using the RID method by Mancini, et al 1965

Statistical Analysis of Data

One way analysis of variance and multiple comparison of means using the Q method (Snedecor and Cochran, 1981) were used to test for significant difference in the levels of immunological parameters (tonsil size, total peripheral lymphocyte count, percent rosetting T-cells) among the subjects grouped according to nutritional status.

The Chi Square analysis was used for the hypersensitivity skin test. For all analysis, $p < 0.05$ level of significance was used.

RESULTS AND DISCUSSION

Delayed Cutaneous Hypersensitivity Test

One test for assessing the cell-mediated immune response in malnourished children, is the delayed cutaneous hyper-sensitivity reaction. This test evaluates the ability of the CMI system to mount a response to a battery of recall antigen such as streptokinase-streptodornase, Candida, trichophyton and the purified protein derivative (PPD). The diameter of induration produced by the intradermal administration of antigen is measured after 48 to 72 hours. A positive reaction should be at least 5 mm in diameter. In this study, the recall antigen used for the skin test was PPD. The reason for the use of this test in this study, is to measure or evaluate the ability of the child's immune system to recall immunological memory of an antigen (BCG) presented during an earlier (BCG) immunization after birth.

Results of the study showed (Tables 2 & 3) that the proportion of PPD-positive children was not significantly reduced in the malnourished groups of children compared to the normal group. The observation holds true for both malnourished subjects grouped according to Waterlow's classification (Table 2) and the FNRI modified Gomez classification (Table 3). These results are in contrast to those of McMurray's et al. (1981) who found the percentage of PPD-positive children to be significantly reduced in the malnourished children.

This study also showed that the induration mean size (Table 3) for the normal, mildly and moderately malnourished children (Using the FNRI weight for age classification) were less than the 5 mm diameter. In contrast, the severely malnourished groups gave an induration mean size greater than 5 mm. equivalent to 6.42 mm. Similar trend was noted when the subjects were grouped according to Waterlow's classification, with the chronic group exhibiting an induration mean size of 5.75 mm (Table 2). These results seem to indicate that there is no significant influence of the nutritional status on the mean size of the induration. However, it is possible that the skin test PPD antigenic dose (4TU) used in this study is not sufficiently strong to elicit a positive response.

Tonsil Size Measurement

Hypertrophy of lymphoid tissues has long been recognized as a hallmark of severe malnutrition and one explanation for impaired immune function. An estimation of tonsil size has been used repeatedly as an easily observable indicator of general lymphoid development. It is admittedly a rather subjective measurement, but one which previous investigators have found to correlate highly with the presence of malnutrition and altered immunological competence in young children (Ziegler and Ziegler, 1975; Symthe, Scholand and Brereton-Stiles, 1971: Neumann, Lawlor and Stiehm, 1975).

TABLE 2. Delayed Hypersensitivity Skin Test (Using PPD) in Filipino Preschool Subjects Grouped According to Nutritional Status (Using Waterlow's Classification)

Nutritional Status (Waterlow's)	N	% Positive Skin Test * (> 5 mm) **	Induration Mean Size
Weight for Height < 85% Height for Age < 90% (Chronic)	8	5/8 or 62.5%	5.75 mm
Weight for Height < 85% Height for Age < 90% (Stunted)	12	4/12 or 33.33%	4.66
Weight for Height < 85% Height for Age > 90% (Wasted)	10	2/10 or 20.00%	3.90
Weight for Height > 85% Height for Age > 90% (Normal)	101	26/101 or 25.74	4.21

* Using 4 TU, PPD (obtained from DOH). Skin test response was observed 48 hours after application.

** Chi Square analysis showed no significant difference between the normal group of subjects vs the three malnourished groups at $p < 0.05$.

TABLE 3. Delayed Hypersensitivity Skin Test (Using PPD) in Filipino Preschool Children Grouped According to Nutritional Status (Using the FNRI-DOST Weight for Age Classification, 1983).

Nutritional Status	N	% Positive Skin Test (> 5 mm Diameter) *	Induration (Mean Size, mm)
Normal	39	9/39 or 23.07%	3.923 = 3.41
Mild	66	17/66 or 25.75%	4.393 = 3.62
Moderate	19	5/19 or 26.31%	4.052 = 2.73
Severe	7	5/7 or 71.42%	6.418 = 2.14

* Chi Square analysis showed no significant difference between the normal group of subjects vs. the three combined undernourished groups at $p < 0.05$.

TABLE 4. Tonsil Size Measurement of Filipino Preschool Children Grouped According to Nutritional Status (Using Waterlow's Classification)

Nutritional Status (Waterlow's Classification)	Mean Tonsil Size (±S.D.)	Number of Children with: No Visible Tonsils (0)	Tonsils Within Faucial Arch (1)	Tonsils Not Beyond Midline (2)	Tonsils Extend Past Midline (3)
Wt/Ht < 85% Ht/Age < 90% (Chronic)	0.75 = 0.66	3/8	4/8	1/8	0
Wt/Ht > 85% Ht/Age < 90 (Stunted)	1.75 = 0.80	1/12	8/12	3/12	0
Wt/Ht < 85% Ht/Age > 90% (Wasted)	1.00 = 0.32	1/10	8/10	1/10	0
Wt/Ht > 85% Ht/Age > 90%	1.54 = 0.47	1/100	43/100	52/100	4/100

* Using the criteria by Symthe et al: Lancet 2: 939-984, 1971. Values significantly different from normal at $p < 0.05$.

TABLE 5. Tonsil Size Measurement in Filipino Preschool Subjects Grouped According to Nutritional Status (Using the FNRI Weight for Age Classification)

Nutritional Status (Weight/Age)	Mean Tonsil Size (±S.D.)	Number of Children with			
		No Visible Tonsils (0)	Tonsils Within Faucial Arch (1)	Tonsils Not Beyond Midline (2)	Tonsils Extend Past Midline (3)
Normal	1.54 = 0.72	2/39	17/39	17/39	3/39
Mild (1)	1.52 = 0.58	2/66	29/66	34/66	1/66
Moderate (2)	1.16 = 0.60	2/22	12/22	5/22	0
Severe (3)	0.71 = 0.75	3/7	3/7	1/7	0

* Using the criteria by Symthe et al: Lancet, 2:939-944, 1971 Significant difference from normal at $p < 0.05$. (ANOVA and Multiple Comparison using the Q Method; Snedecor & Cochran, 1981.

In this study, a significant interaction between nutritional status and tonsillar size was observed (Tables 4 & 5) with a significant reduction of the estimated tonsil size in the undernourished groups of children. This observation suggests that even moderate nutritional deficiency when they occur during this early development period can have a marked influence on the lymphoid development.

Total Peripheral Lymphocyte Count

The mean total peripheral lymphocyte count was found to be significantly different among the subjects grouped according to the FNRI Weight-for age classification (Table 7) but was not significantly different when the subjects were grouped according to Waterlow's classification (Table 6). However, the values obtained were all within normal range and not even one child was found to be lymphophenic (lymphocyte count below 1000 cells/mm3). Noticeably, the lymphocyte count increased with the severity of nutritional status.

Percent Rosetting T-Cells

The total peripheral lymphocyte count consists of both the T-cells which are responsible for the cell-mediated immune response, and the B-cells which are responsible for the humoral response of the host defense system. Although the total lymphocyte count of the subjects in this study are all within normal level regardless of nutritional status, it seems relevant to know which of the subpopulation of lymphocytes are the predominant mononuclear cell population in the blood. The T-cells can be identified by their ability to form rosettes at 4^{0}C with sheep red blood cells (SRBC).

Results showed that the percent rosetting lymphocytes were not significantly different among the subjects grouped according to nutritional status (Tables 8 & 9). The mean value for the percent rosetting capacity of the four groups of subjects in both Tables were quite low compared to those obtained by other workers (Chandra, 1974; Bhaskaram & Reddy, 1974). It is possible that although the total peripheral count of the subjects are within the normal range, the T-lymphocytes subpopulation levels are low as indicated by the low mean values of the percent rosetting lymphocytes.

TABLE 6. Total Peripheral Lymphocyte Count in Filipino Preschool Subjects Grouped According to Nutritional Status (Using Waterlow's Classification)

Nutritional Status (Waterlow's Classification)	N	Total Lymphocyte Count (Cells/mm^3)* *
Weight/height < 85% Height/Age < 90% (Chronic)	8	7666.75
Weight/Height > 85% Height/Age > 90% (Wasted)	12	5973.38
Weight/Height > 85% Height/Age < 90% (Stunted)	11	5630.82
Weight/Height > 85% Height/Age > 90%	106	5546.08

* Normal Range, USA Age Match Standard = 2000 cell/mm^3

** Values are not significantly different from each other at $p < 0.05$.

TABLE 7. Total Peripheral Lymphocyte Count in Filipino Preschool Subjects Grouped According to Nutritional Status (Using the FNRI-DOST Weight for Age Classification, 1983)

Nutritional Status	N	Total Lymphocyte Count (Cells/mm3)
Normal	41	5255
Mild	66	5864
Moderate	20	6262
Severe	7	7559

* Values are significantly different for the four groups at $p < 0.05$ (One-way analysis of variance).

TABLE 8. Percent Rosetting Lymphocytes in the Filipino Preschool Subjects Grouped According to Nutritional Status (Using Waterlow's Classification)

Nutritional Status (Waterlow's Classification)	N	% Rosetting Lymphocytes (Mean = Std. Dev.) **
Weight/height < 85% Height/Age < 90% (Chronic)	8	5.375 + 5.31
Weight/Height < 85% Height/Age > 90% (Wasted)	11	4.70 + 5.31
Weight/Height > 85% Height/Age < 90% (Stunted)	12	8.32 + 8.96
Weight/Height > 85% Height/Age > 90%	106	6.50 + 4.75

$$* \ \% \text{ Rosetting} = \frac{\text{Number of Rosetting Lymphocytes}}{\text{200 Live Lymphocytes}} \times 100$$

** Values are not significantly different from each other at $p < 0.05$ (One Way Analysis of Variance)

TABLE 9. Percent Rosetting Lymphocytes in Filipino Preschool Children Grouped According to Nutritional Status (Using the FNRI-DOST Weight for Age Classification, 1983)

Nutritional Status	N	Percent Rosetting Lymphocytes * Mean = Std. Dev. *
Normal	41	6.08 = 4.64
Mild	66	6.73 = 5.46
Moderate	20	6.57 = 6.22
Severe	7	5.28 = 3.14

$$* \ \% \text{ Rosetting Lymphocytes} = \frac{\text{Number of Rosetting Lymphocytes}}{\text{200 Live Lymphocytes}} \times 100$$

** Values not significantly different for the four groups at $p < 0.05$.

Serum Immunoglobulins (IgG and IgM)

Table 10 and Table 11 show the mean serum immunoglobulin levels in the preschool children grouped according to nutritional status using Gomez classification (as recommended by FNRI-DOST, 1983) and Waterlow's classification respectively. The mean serum IgG level of both the moderately and severely malnourished children were significantly lower than the mean IgG level in the normal subjects (Table 10). The mean serum IgG values of the mildly undernourished children were not significantly different from the normal. Despite the significant low mean IgG values for the moderately and severely malnourished children, the values were all within the normal range of 700-1100 mg/dL set by Neumann et al. (1975) for the USA age-matched standards.

When the children were grouped according to Waterlow's classification (Table 10) no significant differences in the mean serum IgG were noted among the four groups. The mean serum IgG values were also within the normal range.

The mean serum IgM levels were not significantly different among the four groups of subjects in both classification (Table 10 and Table 11). The mean serum IgM values were higher than the reference values, 40-80 mg/dL set for the USA age matched standard by Neumann et al (1975).

The normal serum IgG and elevated IgM values obtained in this study suggest that the humoral immunity was not notably affected or impaired during protein calorie malnutrition. These results agree wwth the observations reported by Munson et al (1974) and Neumann et al (1975). Munson et al reported the high immunoglobulins (IgG, IgM, and IgA) although within normal levels in both the children and adult subjects upon admission. The high immunoglobulin levels were explained to be a response to infection, which eventually normalized when the subjects were discharged. This elevated immunoglobulins in response to infection upon admission and the decrease in the levels after nutritional recovery support the concept that protein calorie malnutrition does not compromise the humoral arm of the defense mechanism. Similarly, Neumann and coworkers also observed elevated immunoglobulin (IgG, IgM and IgA) levels in the three groups studied, with levels somewhat higher in the malnourished groups.

Some parameters of the cell-mediated and humoral

TABLE 10. Mean Serum Immunoglobulins (IgG and IgM) in the Preschool Children Grouped According to Nutritional Status (Using FNRI-DOST Weight for Age Classification)

Nutritional Status	IgG		IgM	
	N	mg/dl	N	mg/dl
Normal	20	902 = 64.59	31	139 = 19.47
Mild (1)	24	879 = 69.56	30	143 = 22.01
Moderate (2)	5	799 = 79.90 **	18	141 = 19.77
Severe (3)	6	823 = 67.29 **	7	133 = 20.74

** Significant difference from normal to mildly undernourished groups at $p < 0.05$.
Normal values in the blood (set by Neumann et al 1975; for USA-aged matched standards)
IgG = 700 - 1100 mg/dl
IgM = 40 - 80 mg/dl

TABLE 11. Mean Serum Immunoglobulins (IgG and IgM) in Preschool Children Grouped According to Nutritional Status (Using Waterlow's Classification)

Nutritional Status	IgG		IgM	
	N	mg/dL	N	mg/dL
Normal > 85 % Wt/Ht > 90% Ht/Age	25	895 = 77.82	25	140 = 17.46
Stunted > 85% Wt/Ht < 90% Ht/Age	8	815 = 74.50	12	131 = 18.75
Wasted < 85% Wt/Ht > 90% Ht/Age	2	785 = 31.82	9	149 = 19.45
Chronic < 85% Wt/Ht	6	823 = 67.29	8	128 = 23.08

* No significant difference in the IgG and IgM values among the four groups at $p > 0.05$

response branches were investigated in the subject population composing of preschool children from an urban depressed community near the UP campus, Diliman, Quezon City. The children were grouped according to nutritional status using the classification by the FNRI-DOST and the Waterlow's classification.

Delayed hypersensitivity skin test using PPD as the response antigen, is a measure of the ability of the child's immune system to recall immunologic memory of the antigen (BCG) presented or introduced during the first six months of the child's life. McMurray et al (1981) found that the percentage PPD positive children were significantly reduced in the malnourished children, indicating an impairment in the ability to mount delayed hypersensitivity skin test.

In this study, no such reduction in the percent PPD positive response were noted in the malnourished children. A mean induration of > 5 mm diameter was noted for the severely malnourished (FNRI-DOST classification) and the chronic group (Waterlow's classification) whereas the less undernourished and normal groups exhibited < 5 mm diameter induration size. There could be two probable explanation for these observations: - first, the nutritional status (particularly, the mild and moderately undernourished groups) may not significantly influence the mean induration size for that matter the delayed hypersensitivity response, and/ or second (which may be the more probable reason), the PPD test antigenic dose of 4 TU used in this study, is sufficient to elicit a positive response. It is interesting to mention that Kielman et al (1975) also found no significant influence of the nutritional status on the mean induration size for reaction greater than 5 mm in BCG-vaccinated Indian children. Mildly and moderately malnourished children who did not react, developed skin tests that were as large as those of their normal cohort. On the other hand, Zeigler and Zeigler found a graded response in induration size with severity of nutritional status. McMurray et al (1981) study have shown that a positive response could be elicited in many children by simply increasing the skin test antigen dose.

The use of a higher PPD antigenic dose in this study, as implied in McMurray et al's investigation may be helpful for future work in order to resolve the effect of the skin test to this particular population of subjects.

A negative skin test does not actually indicate absence of immunologically reactive lymphocytes in the circulation of the individual. To confirm this, the number and blastogenic activity of the peripheral blood lymphocytes are usually also determined. Due to fund constraints, the latter parameter assay (which requires the use of radioisotopes) was not done in this study. The total peripheral lymphocyte count obtained were all within the normal values regardless of nutritional status and not even one child was found to be lyphophenic. This result, in a way, supports the above given premise that the negative skin test (< 5mm induration size) obtained for this subject population (regardless of status) may be due to the very low dose of the PPD antigen used.

The different subpopulation of T-lymphocytes are responsible for the cell-mediated immune response. They can be identified by their ability to bind with sheep red blood cells to form rosettes. Using the rosette-forming assay, Reddy et al (1976) reported significant reduction in the number of T-lymphocytes in Indian children whose body weight were below 70% of the standard. Chandra (1977) also noted a similar diminution in the T-cell number as reflected by reduced % rosettes in underweight children.

In this study, the % rosetting capacity was not significantly different among the four groups in both sets of nutritional status classification. The % rosettes were low compared to the results of the above mentioned workers. This could imply low T-cell subpopulation (despite normal total peripheral blood count) however, the nonsignificance of the % rosetting capacity among the four groups makes it difficult to properly assess or evaluate the validity of the results since even the normal control group also exhibited low% rosette capacity. We suggest further study to confirm this.

A significant reduction in the estimated tonsil size was observed in the undernourished groups in this study, implying that even moderate nutritional deficiency when they occur during the developmental growing period, could markedly affect the lymphoid tissue development. Similar trend was also reported by McMurray (1981).

Protein calorie malnutrition can profoundly affect cellular growth and function especially when it occurs during the early developmental years of life. Every cell type and organ may be affected although at variable degree and time. Organs with high rate of cell renewal, such as the small gut, bone marrow and the lymphoid tissues are easily affected. According to Chandra (1971), the lymphoid tissues in the starved individual can involute significantly. These cellular changes may be the direct result of reduced availability of nutrients necessary for synthesis of cell components or may be mediated through complex alteration in the hormonal balance (ex. raised plasma cortisol in the children with PCM).

No deficiency of serum IgG and IgM was found in undernourished children in this study. On the contrary, the serum IgG and IgM in both the malnourished and normal control groups were higher than the standard values used by Neumann et al. Similar trends were reported by Neumann et al (1976) and Munson et al in their mildly and moderately malnourished subjects: and by Keety and Tham (1969) and marasmic subjects respectively. All these results, imply that the humoral arm of the host defense system is not compromised in protein calorie malnutrition.

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