Comparing nutritional status methods in a Guatemalan survey[1, 2]

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Changes in human hair color and texture are widely used as clinical signs of protein-calorie malnutrition (PCM) (1) and have stimulated the study of hair as an indicator of PCM. Morphological changes in hair roots have been described in severely malnourished children (2–4) and also in experimentally protein-deprived adults (5, 6). In this study, we compared the relationships between two hair-root parameters, mean bulb diameter and percentage atrophy, and the anthropometric and biochemical measurements most frequently used in the evaluation of nutritional status of populations.

Methods

Children 2 to 6 years of age were examined in a clinic on a voluntary basis in two Mayan Indian villages in the Lake Atitlan Basin of the Guatemalan highlands. The sole criteria for inclusion were age and the completion of results in at least two of the three nutritional measurement groups, i.e., anthropometric, biochemical, and hair measurements. All data for this study were compiled at the time of the initial examination of each child. The examinations were completed over separate 3-month intervals in each village; 144 children were studied in San Juan and 35 in Santa Cruz.

The children were weighed on a clinical beam balance and the clothes worn were weighed separately to determine net individual weights. Standing heights were taken against a stabilized measuring rod. Blood and urine specimens were taken after 4 to 5 hr of fasting; the first urine specimen was discarded. A 2-hr thirsting collection was then taken and acidified.

Intraindividual differences in urea/creatinine ratios were minimized by sampling in the morning under thirsting and fasting conditions. The urine and venous blood specimens were kept on ice until both were frozen at −20 C within 5 hr. Hair was epilated with plastic-sheathed forceps from the occipital region and, until examination several months later, the samples were stored in envelopes at room temperature. Chronological ages of the subjects were obtained from official municipal registries.

Plasma amino acids were determined by the method of Whitehead (7) as modified by Arroyave and Bowering (8). Albumin was determined by cellulose acetate electrophoresis and urinary urea nitrogen and creatinine were determined with an AutoAnalyzer. Hair-root diameters were measured with an ocular micrometer and dissecting microscope. Atrophy was expressed as a percentage of hair bulbs in the growing or anagen phase (9). Weight and height ratios were derived from Harvard standards as modified by Jelliffe for combined sexes (1). Each variable was classified by two limits of abnormality: intermediate to low, which included all values below the lower level of normality and represents both borderline and marked abnormality, and low, which was restricted to markedly abnormal values. These classifications were derived from studies of each variable in the literature using Guatemalan data when available. The class limits used are listed in Table 1. The sources used for determining the class limits for each variable were as follows: albumin (10), amino acid ratio (A/A) (11), ratio of urinary urea nitrogen to creatinine (U/C) (12), weight-for-age and weight-for-height (1), hair diameters, and atrophy (2–4).

Results

The prevalence of infectious diseases on the initial examination was similar in both village groups, averaging 24% acute or chronic diarrhea, 3% pneumonia, 3% otitis media, and 13% conjunctivitis. However, 12% had a recent diagnosis of measles or pertussis in San Juan and there was also one case of clinically diagnosed marasmus and three of pre-kwashiorkor in this village.

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The children in the two villages were short for their ages ($\bar{X}$ 83 ± 4%), light in weight-for-age ($\bar{X}$ 71 ± 9%), but normal in weight-for-height obtained ($\bar{X}$ 98 ± 9%). The differences in variables between the villages and the disproportionate numbers studied in each village led us to first seek within-village relationships and then determine which relationships were consistent between villages. The relationships between the two hair measurements and the other variables were observed in several ways: frequency distribution within class limits, chi-square tests for goodness of fit between frequencies, and multivariate canonical correlation analysis.

Table 2 shows the frequency of affected children for each variable in the intermediate to low and low categories in the two villages and the means and standard deviations for each variable. The percentage of children with albumin and A/A ratio levels that placed them in the intermediate to low category was considerably higher in San Juan than in Santa Cruz. There were more children with weight-for-height levels in the intermediate to low category in the former than in the latter. Frequencies for hair-root diameter, atrophy, U/C ratio, and weight-for-age were similar for both villages. Differences between villages reflect both preceding and concurrent dietary intakes and respective experiences with severe infections.

Table 3 lists the chi-square tests. Using the null hypothesis, we found that hair bulb diameter and U/C ratio were not significantly different, i.e., they were related in both the intermediate to low and low categories in each of the two villages. Reduced bulb diameters were also consistently related to reduced weight-for-height in the low categories in both villages. We found that the percentage of hair-root atrophy was not significantly different, i.e., was related to A/A ratio and weight-for-height in both categories and was also consistently related to reduced weight-for-age in the low categories in both villages. Increased percentages of atrophy were related to decreases in serum albumin in Santa Cruz but not in San Juan.

Canonical correlation analysis is a method of examining association among groups of variables. The data of the two villages were combined for this analysis as another method

TABLE 1
Class limits for each variable in the intermediate to low and low categories

Parameter	Class limits	
	Intermediate to low	Low
Diameter (× 10^{-2}mm)	<11	<7
Atrophy, % of anagens	>20	>44
U/C Ratio	<11.0	<6.0
A/A Ratio	>2.5	>3.0
Albumin, g/100 ml	<3.52	<2.80
Weight-for-age, % of standard	<85	<60
Weight-for-height, % of standard	<93	<85

TABLE 2
Frequency (in percent) of affected children for each variable by category and village with number, mean ($\bar{X}$), and SD of variables[a]

	San Juan					Santa Cruz				
	Intermediate to low, %	Low, %	n	$\bar{X}$	±SD	Intermediate to low, %	Low, %	n	$\bar{X}$	±SD
Diameter	53	11	113	11.9	4.9	63	16	19	10.1	4.1
Atrophy	24	2	112	10.0	13.4	26	5	19	10.6	13.8
U/C Ratio	65	10	126	10.3	4.3	57	3	35	11.4	3.9
A/A Ratio	22	3	120	2.14	0.45	9	5	22	2.17	0.33
Albumin	74	33	121	3.16	0.82	23	5	22	3.80	0.59
Weight-for-age	92	6	144	72	9	91	0	35	73	8
Weight-for-height	24	5	144	98	9	43	9	35	96	10

[a] Units of measurement appear in Table 1.

TABLE 3
Comparison of the frequencies within categories of hair-root diameter and atrophy with other variables (chi-square test $H_0: \mu = \mu_0$)

	San Juan				Santa Cruz			
	Intermediate to low		Low		Intermediate to low		Low	
Association	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Diameter with								
Albumin	11.5	<0.01	17.0	<0.01	6.9	<0.01	0.0	0.25
A/A Ratio	24.7	<0.01	4.8	<0.05	13.3	<0.01	0.0	0.25
Weight-for-age	52.2	<0.01	1.6	0.21	0.0	<0.05	0.0	<0.05
Weight-for-height	22.5	<0.01	3.1	0.08	2.0	0.16	0.0	0.35
U/C Ratio	3.5	0.06	0.1	0.84	0.18	0.75	0.0	0.12
Atrophy with								
U/C Ratio	40.7	<0.01	6.5	<0.05	4.7	<0.05	0.0	0.58
Albumin	59.6	<0.01	38.8	<0.01	0.0	0.54	0.0	0.72
Weight-for-age	126.3	<0.01	0.0[a]	0.07	24.2	<0.01	0.0	0.36
A/A Ratio	0.16	0.26	0.0	0.37	0.0	0.15	0.0	0.72
Weight-for-height	0.01	0.96	0.0	0.16	1.4	0.25	0.0	0.56

[a] In cases of blocks less than five, the Fisher Exact Test for one-tailed probability was used. The χ^2 result in such cases was 0.0.

of seeking possible associations between the three heterogeneous groups of variables, hair, biochemical, and anthropometric. The vector in each group that showed the strongest association with the combined variables of each of the other groups were hair bulb diameter, U/C ratio, and weight-for-height.

Discussion

Comparisons among tests of PCM suffer from the lack of an accepted predictor of malnutrition against which each one can be compared. The interpretation of prevalance or cross-sectional surveys is further complicated by a number of factors; i.e., the tests used may show different responses to different types and severity of nutritional stress. Some may reflect nutritional changes more rapidly than others. The nutritional insult may be chronic or acute, and its timing in relation to the time of survey will influence test response. Many of the tests probably change in a curvilinear rather than a linear fashion due to homeostatic mechanisms. Infection and infestation synergistically affect nutritional status. These many factors complicate the methodology and analysis of cross-sectional studies.

In this study, we found a close and consistent relationship between hair-root diameter and U/C ratio. The U/C ratio has been shown to respond quickly to changes in dietary protein intake (13–15). It has also been shown that hair-root diameter and total urinary nitrogen have comparable time-responses during protein deprivation (6). The consistent relationship between root diameter and U/C ratio in this population suggests a similar early and rapid response to altered protein metabolism even though the hair-root changes reflect different metabolic pathways than does the U/C ratio. Hair-root volume, protein, and DNA content are reduced in malnutrition, suggesting a reduction in cell size and number (16). This adjustment is continuous and in the low category, diameter measurements were significantly related to weight-for-height as well. Root atrophy occurs later in the continuum of hair-root changes and is related to loss of protein reserves. This is shown by the consistent relationships between root atrophy and A/A ratio and weight-for-height in the intermediate to low and low categories. A high degree of atrophy or a shift to the resting phase, or both, represents the closing down of an organ system at a cellular level to meet the protein priorities of the total organism.

We conclude that hair-root tissue can be used diagnostically in the assessment of PCM. Hair-root diameter reduction is a good indicator of early PCM for field work. In this study, decreases in hair-root diameter were associated with decreases in U/C ratio, confirming previous work with experimental protein deprivation. Hair-root atrophy is an indicator of more severe or chronic PCM; it is associated with depletion of tissue reserves. Increases in root atrophy were consistently related to depressed weight-for-height and abnormal nonessential to essential serum amino acid ratios in this study.

Summary

Biochemical, hair-tissue, and anthropometric methods used for the early recognition of malnutrition were compared simultaneously in 179 preschool children living in the Guatemalan highlands. Decreases in hair-root diameter and urinary urea/creatinine ratio were consistently related as early indicators of inadequate protein intake. Increased hair-root atrophy was consistently related to increases in the ratio of nonessential to essential amino acids in serum and also to depressed weight-for-height and weight-for-age as later indicators of PCM. Hair-root measurements are useful in determining nutritional status of populations.

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