α_2-Macroglobulin in vitamin A-deficient children[1-3]

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ABSTRACT Serum samples were obtained from 43 children 14 years old or younger in Malaysia and Guatemala. The levels of the serum glycoprotein α_2-macroglobulin (α_2-M) were assayed by two methods: the trypsin-binding assay of Ganrot (Clin. Chim. Acta 14:493, 1966) and a radial immunodiffusion assay against α_2-M antiserum. The two methods gave the same results. When serum α_2-M levels were plotted against serum vitamin A concentrations, they were significantly correlated ($r = 0.505$, $P < 0.001$); children with serum vitamin A levels greater than 40 μg/100 ml had α_2-M levels of 3.71 ± 0.79 mg/ml (mean ± SD, n = 13), while those with levels less than 40 μg/100 ml had α_2-M levels of 2.78 ± 0.51 mg/ml (n = 30); the difference was significant ($P < 0.001$). Normal, apparently healthy children had α_2-M levels of 3.90 ± 0.39 mg/ml. Most of the children sampled suffered from a variety of infections; of these, measles appeared to counteract the effect of vitamin A deficiency by elevating α_2-M levels. Vitamin A-deficient children with measles had α_2-M levels not significantly lower than those of normal children. The difference between deficient and normal values of α_2-M was still significant ($P < 0.05$) when expressed per milligram of serum protein, showing that the effect was not caused by lowered serum protein concentrations associated with protein-calorie malnutrition, from which most of the deficient children suffered. *Am. J. Clin. Nutr.* 32: 1842-1846, 1979.

In the last 20 years, much effort has been expended around the world in an attempt to eliminate vitamin A deficiency in underdeveloped countries. The major stimulus for this effort was the recognition that the incidence of permanent blindness due to corneal ulceration in childhood is often correlated with an episode of severe vitamin A deficiency. That such a permanent disability might be preventable by simply supplementing deficient diets or administering large doses of the vitamin on a regular basis led to the establishment of a number of programs in regions of the world where the deficiency is prevalent. Whether such programs will be successful in decreasing the incidence of vitamin A deficiency is not yet known.

As it is not economically feasible to dose or supplement everywhere, one of the problems in the prevention of vitamin A deficiency is the identification of populations whose intake of the vitamin is marginal. At present, this is done by observing the incidence of ocular signs. The shortcoming of this approach is that a population could show a very low incidence of clinical signs and still have a large number of individuals whose intake of vitamin A is insufficient. In such a population, an epidemic of childhood disease or a natural disaster could precipitate widespread deficiency. This problem could be eliminated if there were a reliable indicator of vitamin A deficiency that is significantly affected before the appearance of ocular signs and that could be measured easily in the field.

Plasma levels of vitamin A or retinol-bind-

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ing protein have been used as indicators of vitamin A deficiency, and are known to correlate with liver reserves of the vitamin, but only in cases of extreme deficiency (1), and cannot, therefore, indicate early stages of depletion. An attempt to correlate levels of urinary vitamin A metabolites with vitamin A status appeared to be unsuccessful (2).

Because the functional levels of the serum protein α_1-macroglobulin (α_1-M) had been shown to decline in vitamin A-deficient rats (3), the present studies were undertaken to determine whether the concentration of the human homologue, α_2-M is lowered in the serum of vitamin A-deficient children. If so, this indicator might be applicable to the early detection of vitamin A deficiency.

Materials and methods

Serum samples for these studies were obtained from the Institute for Medical Research in Kuala Lumpur, Malaysia, and from the Instituto de Nutricion de Centro America y Panama in Guatemala City, Guatemala. The Malaysian samples were accompanied by information on patient age, and serum vitamin A levels were given for deficient patients. The Guatemalan samples were assayed "blind," and the patient data supplied after α_2-M were determined.

The α_2-M concentration of all samples was measured by two different assay methods. One of these was the functional trypsin-binding assay described by Ganrot (4). The standard assay used was scaled down to one-half that reported by Ganrot; conversion of optical density at 410 nm to micrograms of trypsin was by comparison with a standard curve generated with trypsin alone. The second method for determination of α_2-M was radial immunodiffusion. The 1%-agarose plates, prepared in 5 mM phosphate (pH 7.0) containing 0.154 M NaCl and 0.02% (w/v) sodium azide, contained 4% (v/v) antiserum to human α_2-M (2.1 mg antibody per milliliter; Miles Laboratories, Kankakee, Ill.). Human α_2-M solutions (Hyland, Costa Mesa, Calif.), generously supplied by Dr. M. B. Berman of the Eye Research Institute of Retina Foundation in Boston, were used as standards. Serum samples were spotted without dilution (3 μl/hole), and diffusion was for 48 hr at room temperature in a humid chamber. A minimum of two determinations by each assay method was done for each sample.

Serum protein concentration was determined for each sample by the method of Lowry et al. (5). Bovine serum albumin was used to generate the standard curve.

Results

Tables 1 and 2 show the data for serum samples from Malaysia and Guatemala, respectively. While a total of 59 serum samples was received, only 43 of these were from individuals 14 years old or less. The normal

TABLE 1
Serum levels of vitamin A, total protein, trypsin-binding ability, and α_2-M in samples from Malaysia

Patient[a]	Age	Vitamin A	Protein	Trypsin bound	α_2-M
	yr	*μg/dl*	*mg/ml*	*μg/ml*	*mg/ml*
1	4	17	84.8	248	2.98
2	3	11	71.3	180	2.98
3	4	20	87.0	272	3.17
4	4	20	93.0	308	3.42
6	6	4	66.8	252	2.60
7	6	17	80.3	180	1.83
8	8	12	96.0	228	2.35
9	10	5	87.8	244	2.38
11	4	8	62.3	204	2.09
12	6	23	100.5	308	2.83
13	2	8	74.3	230	2.35
113	14	NG[b]	82.5	322	3.37
114	11	NG	93.8	360	4.00
115	12	NG	89.3	344	4.30
116	6	NG	86.3	276	3.45
118	6	NG	83.3	358	4.05
119	2	NG	91.5	342	4.22

[a] Patients 1 to 13 were vitamin A deficient, patients 113 to 119 were "normal, apparently healthy subjects." [b] Information not given.

TABLE 2
Serum levels of vitamin A, total protein, trypsin-binding ability, and α_2-M in samples from Guatemala

Patient	Age	Vitamin A	Protein	Trypsin bound	α_2-M
	mo	*μg/dl*	*mg/ml*	*μg/ml*	*mg/ml*
B4620	10	8.3	83.3	244	2.40
B4640	48	36.6	90.0	260	3.09
B4755	24	7.1	75.8	256	2.49
B4777	36	8.3	72.0	328	3.75
B4783	60	38.9	80.3	256	2.56
B4853	36	41.3	82.5	354	4.00
B6083	2	45.2	82.2	214	3.77
B6106	10	8.9	53.5	224	2.97
B6108	9	30.6	65.1	304	3.23
B6811	3	47.7	84.5	193	2.39
B6818	29	14.9	64.3	232	2.90
B6821	8	40.7	98.4	407	5.40
B6824	10	9.4	62.8	203	2.52
B6826	20	43.8	72.1	273	3.39
B6830	7	31.3	85.3	278	2.62
B6837	50	4.7	41.9	172	2.38
B6845	5	15.6	58.9	314	3.80
B6857	81	15.6	82.2	154	2.17
B6862	81	43.8	77.5	232	2.50
B6863	70	33.6	95.3	193	2.45
B6865	15	15.6	64.3	170	2.08
B7045(M)[a]	37	7.0	95.3	243	3.00
B7046	36	59.4	94.6	309	3.33
B7178(M)	26	7.0	112.4	301	3.23
B7421(M)	39	6.3	89.1	308	3.52
B7440(M)	24	13.3	72.9	296	3.28

[a] Patient with measles.

concentration of α_2-M decreases in adulthood (6), a fact we were able to confirm. Therefore, samples from children only could be used for the comparison of α_2-M concentrations in vitamin A-deficient and normal sera. This severely limited the amount of data that could be collected on "normal" individuals, as it is difficult to obtain serum samples from such children in underdeveloped countries.

Figure 1 shows the correlation between the two methods used to determine α_2-M concentrations in serum. In agreement with Ganrot (4), the two assays gave essentially the same results. Outside of experimental error, some of the variability could be due to the fact that serum samples were frozen for storage and transport. Freezing and thawing could have an effect on the concentration of α_2-M as measured by either assay method (M. B. Berman, personal communication), but the effect would not necessarily be the same for both methods. It is preferable with respect to α_2-M stability to lyophilize serum samples as soon as they are collected; this is not usually feasible in the field, however.

When the α_2-M data from children (Tables 1 and 2) were plotted against serum vitamin A concentrations, linear regression by the least-squares method showed them to be significantly correlated ($r = 0.505$; $P < 0.001$) (Fig. 2A).

Figure 3A compares the serum α_2-M concentrations of children with serum vitamin A concentrations more than 40 μg/100 ml

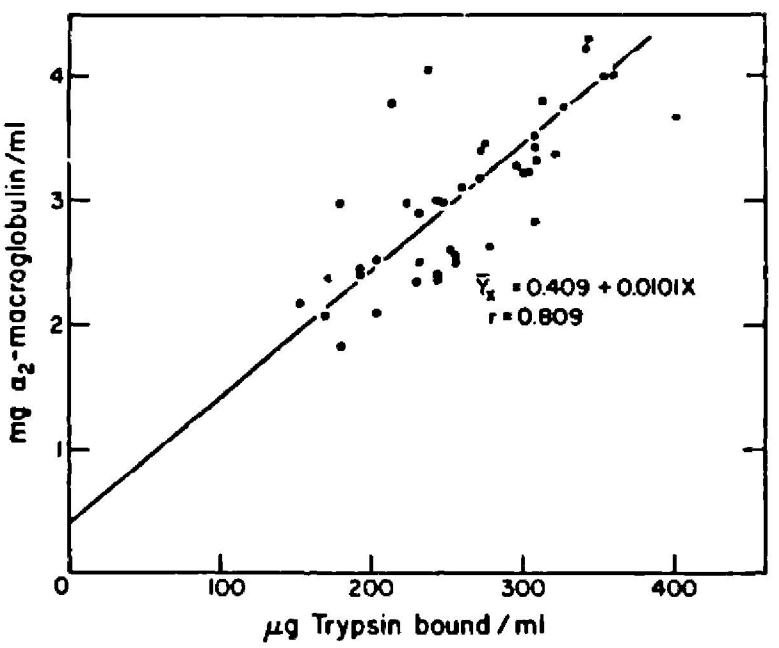

FIG. 1. Comparison of results of the radial immunodiffusion assay for serum α_2-M (described in "Methods") with those of the trypsin-binding assay (Ganrot (4)).

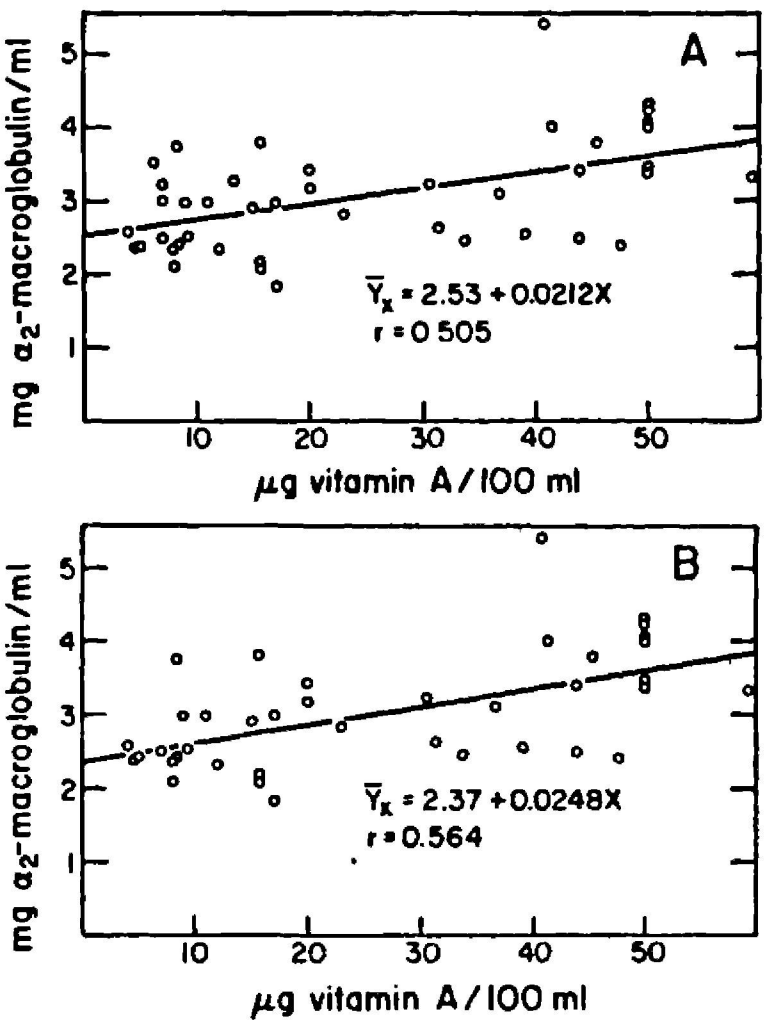

FIG. 2A. decline in serum α_2-M levels correlated with decline in serum vitamin A levels of all individuals from Malaysia and Guatemala (data from Tables 1 and 2). Linear regression was plotted by the least-squares method. B, the same data plotted without the four individuals with measles

("normal")* with those of children with vitamin A concentrations less than this level; the former had an average α_2-M concentration of 3.71 ± 0.79 mg/ml (mean ± SD; n = 13), and the latter had an average concentration of 2.78 ± 0.51 mg/ml (n = 30); the difference in means was significant ($P < 0.001$). Comparison on the basis of trypsin-binding ability was also significant (normal: 297 ± 65 μg/ml, deficient: 246 ± 49 μg/ml; $P < 0.005$).

It is probable that at least five of the normal Guatemalan children (Table 2) had received some type of vitamin A dosing before the serum samples were collected, as they all had conjunctival xerosis, and two showed more advanced ocular degeneration (one had cor-

* This value is an arbitrary cut-off point. For comparison, Smith et al. (8) show this to be the serum vitamin A level found after vitamin A dosing of children suffering from vitamin A deficiency and protein-calorie malnutrition in Thailand; Gomez et al. (9) report a level of 36.3 ± 13.7 (SD) μg/100 ml in normal preschool children in northeast Brazil.

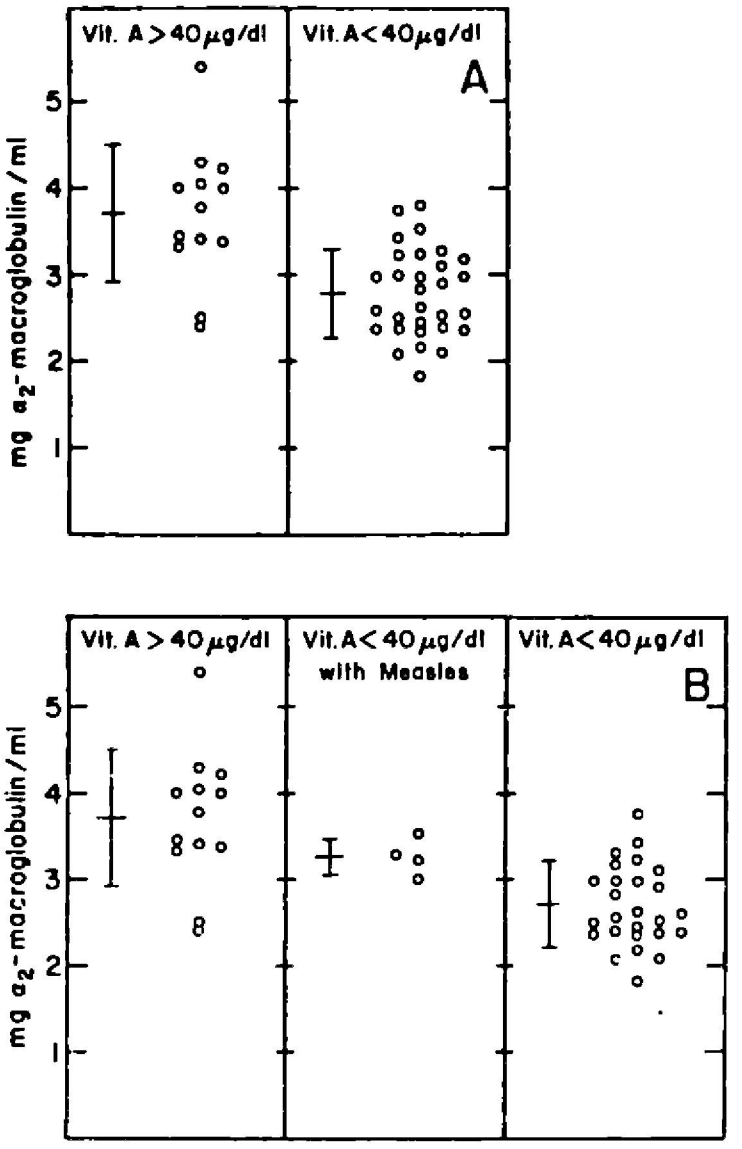

FIG. 3A. comparison of individual serum levels of α_2-M (assayed by radial immunodiffusion, as described in "Methods") of children from Guatemala and Malaysia (data from Tables 1 and 2) with serum vitamin A levels greater than 40 μg/100 ml (n = 13) with those having serum vitamin A levels less than 40 μg/100 ml (n = 30). (For the purpose of this comparison, the normal Malaysian children were assumed to have serum vitamin A levels above 40 μg/100 ml.) B, the same data with individuals with measles and serum vitamin A levels below 40 μg/100 ml (n = 4) plotted separately.

neal xerosis, the other had corneal ulceration). No information was available as to the amount or time of dosing. It was assumed that sufficient time had elapsed for α_2-M levels to recover, even though this was probably not the case for all of these individuals. As a result, the average α_2-M level of all normal children (3.71 mg/ml) is somewhat lower than the result obtained when only data from the six "normal, apparently healthy" Malaysian children were averaged (3.90 ± 0.39 mg/ml).

Discussion

α_2-M levels in serum of children suffering from vitamin A deficiency were found to decline. Since information was available on the general health of the Guatemalan patients, we found that most of the children investigated had multiple ailments in addition to vitamin A deficiency and protein-calorie malnutrition, such as diarrheal syndromes, electrolyte imbalance, bacterial dysentery, bronchopneumonia, bronchial asthma, measles, tonsillitis, and conjunctivitis. Nevertheless, the only condition that appeared to counteract the assessment of vitamin A deficiency by elevating the α_2-M levels was measles. For children with this disease and plasma vitamin A levels less than 40 μg/100 ml (indicated in Table 2), the α_2-M concentration averaged 3.26 ± 0.21 mg/ml (n = 4), significantly higher than the average for the remaining vitamin A-deficient children (2.71 ± 0.50 mg/ml; $P < 0.025$): the vitamin A-deficient children with measles had α_2-M levels not significantly lower than those of the normal children ($P > 0.1$) (Fig. 3B). Eliminating data from vitamin A-deficient children who also had measles from the calculation of the correlation between serum vitamin A concentration and α_2-M levels raised the correlation coefficient from $r = 0.505$ to $r = 0.564$ (Fig. 2B).

Since children with vitamin A deficiency usually suffer from some degree of protein-calorie malnutrition as well, it is possible that the lower serum levels of α_2-M in such individuals were simply due to the lower total serum protein concentrations associated with protein-calorie malnutrition (7). While there was a correlation between total serum protein concentration, these correlations were significant only at $P < 0.1$. In addition, six children with protein-calorie malnutrition who still had serum vitamin A levels above 40 μg/100 ml had an average α_2-M concentration of 3.46 mg/ml, well above the level for the vitamin A-deficient children. When values were expressed as micrograms of α_2-M per milligram of serum protein, the magnitude of the difference between normal and deficient values was decreased, but the difference was still significant (normal: 43.0 ± 7.5 μg/mg, deficient: 37.0 ± 10.4 μg/mg; $P < 0.05$). Taken in total, then, the evidence would indicate that the decline in α_2-M concentrations in the vitamin A-deficient children was due to the deficiency and not to protein-calorie malnutrition.

While the results found in vitamin A-deficient children are in agreement with those obtained in rats (3), it is difficult to judge, based on the evidence obtained in the present studies, whether the measurement of α_2-M levels in serum could be exploited as a method for the early detection of vitamin A deficiency. With only six serum samples from normal, healthy children, the normal range of α_2-M concentration cannot as yet be accurately assessed. Even if the range was ultimately found to be narrow, the opposing effect of certain inflammatory conditions on α_2-M concentration might limit the reliability of the assay. It is clear, however, that further investigation is needed before a decision can be made as to the usefulness of this method.

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