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Lack of Direct Association between Serum Transferrin and Serum Biochemical Indicators of Vitamin A Nutriture

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Key words: Vitamin A - RBP - Transferrin - Iron - Vitamin A-iron interaction.

SUMMARY

The association between serum transferrin and serum biochemical indicators of vitamin A and protein nutriture was investigated in 295 preschool children. Their levels of retinol and RBP correlated positively and significantly with transferrin, iron, total iron binding capacity (TIBC), total proteins and albumin. However, when removing those cases having unacceptable levels of total proteins (< 6.4 g/dl) and/or albumin (≤ 3.5 g/dl), the significant correlation between retinol (or RBP) and transferrin disappeared. It is concluded that transferrin levels are not directely related to the vitamin A nutritional status. The significance of this observation in relation to the known interaction between vitamin A and iron is discussed.

RIASSUNTO

Mancanza di una correlazione diretta tra transferrina serica e indicatori biochimici dello stato nutrizionale per la vitamina A.

In 295 bambini di età prescolare si è valutata la possibile associazione tra gli indicatori dello stato vitaminico A e i livelli serici di transferrina. I livelli di retinolo e *Retinol Binding Protein* (RBP) erano correlati significativamente e positivamente con quelli del ferro, della transferrina, della capacità totale di legare il ferro (TIBC), delle proteine totali e dell'albumina. Eliminando però i casi con valori inaccettabili di proteinemia totale (< 6,4 g/dl) e/o di albuminemia (< 3,5 g/dl), la correlazione scompariva. Si conclude che i livelli di transferrina non sono significativamente correlati con lo stato vitaminico A. Si discute il significato di questa osservazione in relazione alla nota interazione tra vitamina A e ferro.

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INTRODUCTION

Several studies have indicated a biological interaction between vitamin A deficiency and iron nutrition and metabolism (1-5). Recently, Mejía and Arroyave (6) have shown that the national program of sugar fortification with vitamin A in Guatemala brought about a significant improvement, not only in vitamin A nutriture, but also in the iron nutritional status of preschool children. Six months after fortification began it was observed that despite a significant increase in serum iron levels there was also a concomitant elevation in the levels of total iron binding capacity (TIBC). Since TIBC measures indirectly the levels of the iron carrier protein transferrin, this observation suggested that hypovitaminosis A may negatively affect the levels of transferrin, this phenomenon becoming a limiting factor on iron transport. The present study was undertaken to investigate whether there was an epidemiologic association between serum transferrin (measured directly) and serum biochemical indicators of vitamin A nutriture in children, and to what extent this association was related to the protein nutritional status.

METHODS

The study was performed using stored serum samples from INCAP's serum bank. The samples used corresponded to rural preschool children aged 12 to 72 months; they were collected in El Salvador in 1976 to assess the vitamin A nutritional status of that population. In order to have a wide range of serum retinol values, the samples were selected based on their already known retinol levels and classified in three groups as follows: Group I (n = 94), retinol < 20 μg/dl; Group II (n = 105), retinol 20-30 μg/dl; and Group III (n = 96), retinol > 30 μg/dl. These retinol values had been previously determined by the spectrophotometric method of Bessey et al. (7). In the present study, the following additional biochemical parameters were determined: retinol binding protein (RBP) and transferrin by radial-immunodiffusion as described by Mancini *et al.* (8); iron, TIBC and percent transferrin saturation (% TS) by the method of Ramsay (9); ferritin by the two-site radioimmunometric analysis described by Miles *et al.* (10); and total proteins and albumin by zone electrophoresis using a cellulose acetate support medium (Beckman Microzone cell Model R-101 attached to a Beckman Microzone scanning device Model R-102).

The data obtained from the three groups of children were statistically analyzed by analysis of variance and the specific differences between groups by linear contrast (11). Linear correlations were also perfomed in the total group (n = 295) between transferrin and indicators of vitamin A nutritional status (retinol and RBP) and between transferrin and indicators of protein nutriture (total protein and albumin). The effect of removing from the total sample those children with unacceptable protein nutritional status (total protein ≤ 6.4 g/dl and/or albumin < 3.5 g/dl) was also investigated. These values are the cut-off points below which these biochemical parameters are considered in the low and deficient range and thus indicative of protein undernutrition (12). Furthermore, since not only protein but also iron nutriture is known to affect transferrin levels, the removal from the sample of children considered iron-deficient was also tested. This was done by eliminating individuals with serum ferritin less than 7 ng/ml that according to Siimes *et al.* (13) is the level below which children of this age have

depleted iron stores.

It is known that, in iron deficiency, the levels of TIBC and transferrin become increased as a natural physiological response. In order to evaluate the role of vitamin A nutritional status, this phenomenon was tested in children with variable retinol levels but who were iron-deficient (ferritin < 7 ng/ml) and had no limitation on protein nutrition (albumin > 3.5 g/dl). In this selected group (n = 75) the response to the challenge of being iron-deficient was evaluated by correlating serum retinol versus transferrin levels.

The level of α to define statistical significance was set at $p < 0.05$ for all statistical analyses.

RESULTS

Table I shows the average levels of serum biochemical indicators of vitamin A, iron and protein nutriture in the children classified according to their serum retinol. As expected, serum RBP was significantly different in the three groups in accordance with the increasing different levels of retinol. Significant differences were also found in the levels of transferrin, iron, % TS, total protein and albumin. Serum transferrin was significantly higher in Groups II and III than in Group I. Serum iron and % TS were higher in Group III than in Groups I and II. The levels of total protein and albumin were also significantly different among the three groups, in a similar fashion as RBP and retinol.

The correlations between serum biochemical indicators of vitamin A and iron and protein nutriture are shown in Table 2. In the total sample (n = 295), the levels of transferrin, iron and TIBC correlated significantly with both retinol and RBP levels. Likewise, total protein and albumin correlated with RBP. However, when children having unacceptable levels of protein (≤ 6.4 g/dl) or albumin (≤ 3.5 g/dl) were removed from the sample, the previously observed significant correlations between transferrin or TIBC and retinol or RBP disappeared (Table 3). This was not the case for the correlations observed between serum iron versus retinol or RBP where, despite the removal of the subjects with inadequate albumin levels, the correlations remained at a significant statistical

Table 1 - Serum biochemical indicators of vitamin A, iron and protein nutriture in children classified according to their retinol levels.

	Group I	Group II	Group III
	(n = 94)	(n = 105)	(n = 96)
Retinol (µg/dl)	< 20 (14.3 ± 3.7*[a])	20 – 30 (24.8 ± 3.0[b])	> 30 (36.5 ± 7.8[c])
RBP (µg/ml)	20.5 ± 5.1[a]	26.8 ± 3.5[b]	34.4 ± 6.4[c]
Transferrin (mg/dl)	272.7 ± 57.3[a]	291.8 ± 43.6[b]	295.7 ± 48.2[b]
Iron (µg/dl)	54.0 ± 18.3[a]	57.7 ± 20.8[a]	67.1 ± 23.7[b]
TIBC (µg/dl)	321.5 ± 66.9[a]	334.9 ± 53.6[a]	333.6 ± 57.9[a]
% TS	17.9 ± 8.0[a]	17.7 ± 7.2[a]	20.8 ± 8.5[b]
Ferritin (ng/ml)	13.1 ± 10.5[a]	11.5 ± 7.8[a]	13.7 ± 7.6[a]
Total protein (g/dl)	6.7 ± 0.9[a]	7.0 ± 0.5[b]	7.3 ± 0.7[c]
Albumin (g/dl)	3.8 ± 0.6[a]	4.2 ± 0.4[b]	4.4 ± 0.4[c]

* $\bar{x}$ ± S.D.
Mean Values with different superscript letters are significantly different.
($p < 0.05$ or better).

Table 2 - Correlation coefficients among biochemical indicators of vitamin A, iron and protein nutriture.

	Retinol (μg/dl)	RBP (μg/ml)
Transferrin (mg/dl)	0.22*	0.31*
Iron (μg/dl)	0.21*	0.23*
TIBC (μg/dl)	0.13*	0.23*
% ST	0.10	0.08
Ferritin (ng/ml)	0.07	0.04
Total proteins (g/dl)	0.36*	0.40*
Albumin (g/dl)	0.50*	0.53*

* $p < 0.05$, ($n = 295$).

Table 3 - Correlation coefficients among biochemical indicators of vitamin A and iron nutriture in children with acceptable levels of albumin (> 3.5 g/dl) and ferritin (≥ 7.0 ng/ml).

	Retinol (μg/dl)	RBP (μg/ml)
Transferrin (mg/dl)	0.08	0.13
Iron (μg/dl)	0.21*	0.23*
TIBC (μg/dl)	– 0.04	0.01
% ST	0.19*	0.20*
Ferritin (ng/ml)	0.03	0.04

* $p < 0.05$, ($n = 192$).

level (shown in the same table). The correlation with % TS also became significant, probably due to the fact that by removing unacceptable albumin and ferritin levels, the group became more homogeneous and protein and iron nutriture were no longer limiting factors.

Furthermore, when the levels of serum transferrin and retinol were correlated in the group of children who had acceptable levels of albumin (> 3.5 g/dl) but were iron-deficient (ferritin < 7 ng/ml) only a poor non-significant association was found ($r = 0.19$, $p > 0.05$).

DISCUSSION

Vitamin A deficiency is known to impair the synthesis of some glycoproteins (14). Since transferrin is a glycoprotein, it is reasonable to suggest that the lack of vitamin A may directly impair its synthesis and thus explain the epidemiologic association, previously observed in children, between TIBC levels and the levels of serum retinol (6). In the present study, the analysis of the whole sample revealed that children with high retinol and RBP levels (Group III) also have higher levels of transferrin than those in the low range of retinol values (Groups I and II (Table 1). Furthermore, there was a significant correlation between transferrin and retinol or RBP, indicating an association between these parameters (Table 2). This single observation would suggest that transferrin levels are directly related to the vitamin A nutritional status. However, when children with inadequate protein nutriture are removed from the whole group, such significant association no longer exists. This means that transferrin levels in these children depend on their protein nutritional status rather than on their vitamin A nutriture. Further support for this conclusion is given by the result obtained when correlating serum retinol versus transferrin in those children who had acceptable levels of albumin (> 3.5 g/dl) but were iron-deficient (ferritin < 7 ng/ml).

It is well established that in iron deficiency the levels of TIBC, and consequently those of transferrin, become elevated. Based on this phenomenon, one may expect that if indeed the vitamin A nutritional status affects the levels of transferrin, children with no limitation in their protein nutriture (adequate albumin levels) should respond to the challenge of iron deficiency according to their retinol levels. In other words, those children with low retinol should respond poorly to the expected increase in transferrin, and those with higher levels of retinol should also have higher levels of this protein. If this were the case, a

strong significant correlation between these two paramenters should have been found in the latter analysis. However, only a weak no-significant association was found in this subgroup of children ($r = 0.19$, $p > 0.05$), indicating that when the protein nutritional status is adequate, there is no relation between vitamin A nutriture and the levels of transferrin.

Several studies have shown that there is a significant epidemiologic association between the serum levels of retinol and iron (2, 6, 15). It is interesting to observe in the present study that even when removing from the sample those children with inadequate protein nutritional status, the association between retinol or RBP and iron or % TS still holds and remains at a significant statistical level. Protein nutriture had never been considered in previous investigations of this nature and this observation supports the known interaction between hypovitaminosis A and iron nutrition and metabolism (6). It indicates that such interrelationship is not related to the protein nutritional status or to any specific effect that hypovitaminosis A may have on the levels of the iron carrier protein transferrin.

The present data do not allow to elucidate why the children in our previous study showed an increase of TIBC levels when the vitamin A nutritional status improved through sugar fortification (6). One can only speculate that it may be a more general effect of vitamin A on protein utilization and metabolism.

Through animal experiments it is known that vitamin A deficiency can affect nitrogen balance, suggesting an impaired utilization of protein (16). It is also known that the lack of this vitamin negatively affects protein synthesis by impairing the synthesis of RNA (17). Although these aspects of protein metabolism have not been studied in humans, it is reasonable to think that vitamin A may favor protein nutriture which in turn improves the plasma protein levels including transferrin, and consequently TIBC.

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