Hematologic Effect of Vitamin A Supplementation in Anemic Pakistani Children

Pages with reference to book, From 34 To 38

Imrana Khan (Department of Biochemistry, Dow Medical College, Karachi.)
Abdul Baseer (Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi.)

Abstract

To assess the prevalence of vitamin A deficiency in anemic Pakistani children and investigate the hematologic response to vitamin A supplementation, 4 8 year old primary school children from the slum areas of Karachi were surveyed for anemia Of 101 anemic children selected, 16% had low level of vitamm A (<20 ug/dl) and an addilional 2% had deficient level (<10 ug/dl) Serum Retinol level showed positive associated with serum iron, ferritin, hemoglobin, hematocrit and Mean cell hemoglobin concentration. A non-randomized control trial was then carried out. Oral vitamin A capsules were given to 42 children and 53 children served as controls. After 6 weeks, there were significant differences between the two groups for Retinol, Retinol-Binding-Protein and Hematocrit. However, no significant difference could be found for Hemoglobin, RBC count, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration, Serum iron, ferritin or transferrin. A single vitamin A supplement improved the hematocrit in 6 weeks. Long-term studies are needed to find if the WHO recommended periodic massive doses of vitamin A besides improving the morbidity and mortality will also improve the overall picture of anemia in children (JPMA 46:34, 1996).

Introduction

Vitamin A deficiency is endemic in large parts of Africa and Asia with scattered foci in the Carribbean, the Near East and Latin America[1]. It is not only associated with eye damage and a higher childhood moibidity and mortality but also an anemia, resembling iron deficiency anemia sets in[2-5]. Vitamin A supplementation has been shown to improvethisanemia[5-11]. In 1982, Douer and Koefflcr showed that Retinoic acid (a metabolite of vitamin A) enhances the growth of Human early erythroid progenitor cells in vitro[12].

In Pakistan, nutritional anemia is widely prevalent, the most common of which is iron deficiency anemia[13-15]. A few scattered studies show that Vitamin A deficiency is also present. Since the racial, dietary, socioeconomic and geo¬graphical pattern of our children differs from their peers elsewhere, this study was designed to fifld the prevalence of vitamin A deficiency in anemic primary school children belongmg to low socio-economic strata and to investigate the response to a single oral vitamin A supplement.

Subjects and Methods

Primary school children between the ages 4 to 8 years and belonging to Liyari, a low socio-economic area of Karachi, were screened for anemia on an electric hemoglobin photometer (Electroluxmecatronik, Aktiebolaget Leo Diag¬nostics, Helsingborg, Sweden). Anemic children were se¬lected who met the WHO (1988) criteria for anemia i.e., 4-5 years old with Hemoglobin <11 g/dl and 5-8 year old with Hemoglobin <12 g/dl. Out of 19% children, thus screened, 133 were found anemic and of these 120, who were not suffering from any infections (fever, cough or diarrhea) at the

time of examination, were eligible for the study. The study protocol. was explained to the parents and their wntten consent taken.
The study protocol was also approved by our institutional human rights committee. The children's eyes were examined with a hand torch. They were weighed and theirleft mid-upper arm circumference (MAC) and height noted. 5 ml of venous blood was obtained. Alternate children were then assigned to the supplement group and placebo group.
The children belonging to the flrst group received the vitamin A supplement in the form of one capsule of 3 drops of an oily solution containing 200,000 I.U. of vitamin A+40 I.U. vitamin E (obtained from UNICEF). The capsule nipple was snipped off and the contents emptied on the child's tongue. The childrenbelonging to the latter group, received 3 drops of olive oil in a similar way. Six weeks later, the blood sampling was repeated. However, 19 subjects were lost due to failure of follow-up or hemolysis of blood sample. The study was conducted on 101 children. None of the subjects reported any significant treatment complications.
Biochemical analyses 0.5 ml of blood was immediately mixed with 0.5 mg EDTA powder and assayedwithin24 hours onSysmexK 1000 hematology auto-analyser for hemoglobin, hematocrit, RBC count, Mean corpuscular hemoglobin, Mean corpuscular volume and meancorpuscular hemoglobin concentration. The rest of the blood was allowed to clot and then centrifuged to separate the serum. The following biochemical analyses were carried out on the serum:- Retinol by High-precision liquid -chromatography (HPLC)[16], Retinol-binding protein, Prealbumin and Transferrin by the single radial immunodiffusion technique[17] (with the relevant specific antiserum obtained from Behringwerke AG Diagnostica, Mathurg, FR.G.). Ferritin by enzyme based immunometric luminescent technique[18] (Amerlite analyzer and reagents obtained from Amerlite Diagnostic Ltd., Mandeville House, 62 the Broad- way, Amersham, Buckinghamshire, England), iron by atomic-absorption spectrophotometiy[19] (AAS model 3030 B, Perkin Elmer Co., Norwalk, CT) and albumin by Bromcresol green method[20] on Hitachi 705 automated analyzer.
Statistical analysis
In the baseline survey, simple correlations were investigated between Retinol and bi hemlcal indicators of the iron status.
In the intervention trial, the effect of treatment was analysed. Means and standard errors of differences of the measurements at 0 and 6 weeks for the Control and Supplemented groups are presented.

Results

A baseline survey

At base line, the mean age of 53 cases in the control group was 6.13±0. 19 years with an annual per capita income of Rs.2617.85±176.20. In the 48 to be supplemented group, the mean age was 6.42±0.21 with annual per capita income of Rs.2350.48±166.78. There was no significant difference in the age, sex, anthropometric indices, nutritional status or annual per capita income between the control or supplemented groups. Similarly the biochemical indicators of iron and Vitamin A status were also the same in both groups (Tables I, II,III and IV).

Table I. Baseline comparison of anthropometric indices and nutritional status of the control and supplemented children.

	Control (53)	To be supplemented (48)
Height (cm)	110.86±1.96	110.98±1.84
Weight (kg)	17.34±0.61	17.52±0.59
Mid Arm circum-ference (mm)	156.34±1.81	156.56±1.76
Normal (%)	17.0	20.8
Nutrition (%)		
Wasted	26.4	14.6
Stunted	13.2	12.5
Wasted and stunted	43.4	52.1

($P>0.05$ in all parameters i.e. no significant difference between control and supplemented groups at baseline).

Values are reported as Mean and Standard error of mean .

Table II. Baseline comparison of biochemical indicators of vitamin A and iron status in control and supplemented children .

	Controls (53)	To be supplemented (48)
Retinol (µg/dl)	31.25±2.02	27.34±1.35
Retinol binding protein (mg/dl)	21.77±2.98	16.79±0.81
Prealbumin (mg/dl)	14.09±0.98	13.64±0.75
Iron (µg/dl)	44.42±2.89	42.30±3.63
Ferritin (ng/ml)	12.00±1.78	10.73±1.76
Transferrin (g/l)	3.26±0.13	3.47+0.19

($p>0.05$ in all parameters i.e., no significant difference between control and supplemented groups at baseline).

Values are reported as Mean and Standard error of Mean..

Table III. Baseline comparison of erythrocyte indices in control and supplemented children.

	Control (53)	To be supplemented (48)
Hemoglobin (g/dl)	9.94±0.19	9.71±0.28
Hematocrit (%)	30.92±0.46	29.98±0.66
RBC count ($x10^6$/ml)	4.90±0.08	4.70±0.11
Mean corpuscular volume (fl)	64.41±1.31	64.71±1.52
Mean corpuscular hemoglobin (pg)	20.92±0.52	21.01±0.66
Mean corpuscular hemoglobin concentration (g/dl)	32.37±0.30	32.20±0.38

($P>0.05$ in all parameters i.e. no significant difference between control and supplemented groups at baseline).

Values are reported as Mean and Standard error of Mean.

Table IV. Baseline correlation coefficients between retinol and biochemical indicators of iron status.

Variable		No. of children	Value	Coefficient coefficient with Retinol (r= value)
Retinol	(µg/dl)	101	29.39±1.25	
Hemoglobin	(g/dl)	101	9.83±0.17	0.1683
Hematocrit	(%)	101	30.47±0.40	0.1061
RBC count	(10^6/µl)	101	4.81±0.07	0.0581
Mean Corpuscular Volume	(fl)	101	64.55±0.99	0.0235
Mean Corpuscular Hemoglobin	(pg)	101	20.96±0.41	0.1026
Mean cell hemoglobin concentration	(g/dl)	101	32.29±0.24	0.2473**
Albumin	(g/dl)	101	4.87±0.04	-0.1511
Iron	(µg/dl)	101	43.43±2.27	0.2884**
Ferritin	(ng/ml)	101	11.40±1.24	0.3255**
Transferrin	(g/l)	22	3.37±0.12	-0.0853
%Saturation of Transferrin	(%)	22	10.96±1.63	0.0883
RBP	(mg/l)	22	19.28±1.60	0.2316
Prealbumin	(mg/dl)	22	13.86±0.60	0.1949

**Significantly correlated ($p<0.01$)

Distribution of vitamin A deficiency in both sexes revealed that of 43 male children 2.3% were deficient (<10ug/dl), 16% had low levels (10-20ug/dl) and 81.4% had adequate levels. Of 58 females, 1.7% had deficient, 15%low and 82.8% adequate levels. The vitamin A deficiency was slightly higher amongst boys. Table IV shows the baseline correlation coefficients between Retinol and biochemical indicators of iron status. Retinol is significantly though weakly correlated with Mean Corpuscular Hemoglobin concentration (MCHC) ($r=0.2473$ and $p<0.01$), serum iron ($r=O.2884$ and $p<0.01$)and Fernt n ($r=0.3255$ and $p<0.01$).

Table V. Multiple regression equations with hemoglobin (Hb), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and transferrin.

Independent Variables	Dependent Variables Hb (g/dl)	HCT (%)	MCH (pg)	MCHC (g/dl)	Transferrin (g/dl)
r-constant	1.624	2.049	14.373	23.373*	2.149
	(3.776)	(9.417)	(13.783)	(5.824)	(1.992)
Age (years)	0.612*	1.138*	1.181	0.733*	0.011
	(0.161)	(0.403)	(0.590)	(0.249)	(0.085)
Sex	0.817	2.515	0.009	0.619	0.093
	(0.522)	(1.302)	(1.905)	(0.805)	(0.275)
Retinol (µg/dl)	0.077*	0.182*	-0.017	0.102*	-4.736
	(0.033)	(0.083)	(0.122)	(0.051)	(0.017)
Albumin (g/dl)	0.903	2.595	0.162	0.126	0.211
	(0.726)	(1.812)	(2.652)	(1.120)	(0.383)

*P<0.05

B-Intervention trial

The nutritional status and anthropometric indices of the children in the control and supplemented groups, was the same in the baseline survey.

The table shows the regression coefficient with standard error in parentheses.

Table V presents the results of multiple regression analyses. Retinol was significantly positively associated with Hemoglobin, Hematocrit and MCHC. An increase of 1 ug/dl Retinol was accompanied with 0.077 g/dl increase of hemo g1obin~ 0.182% increase inhematocrit and 0.102 gIdl increase in MCHC, when adjusted for difference in age, sex and albumin level.

Table VI. Comparison of biochemical and hematologic variables in the control and vitamin A supplemented groups.

Indices	No of children+	Baseline	6 weeks	Difference between baseline and 6 weeks
Hemoglobin (g/dl)	C (53)	9.94±0.19	9.81±0.19*	-0.13±0.06
	S (48)	9.71±0.28	9.67±0.28	-0.04±0.08
Hematocrit (%)	C (53)	30.92±0.46	32.11****±0.45	1.19±0.22
	S (48)	29.98±0.66	31.95****±0.64	1.97#±0.25
RBC count (10^6/µl)	C (53)	4.90±0.08	4.84±0.08	-0.06±0.03
	S (48)	4.70±0.11	4.73±0.10	0.03±0.04
Mean Corpuscular Volume (fl)	C (53)	64.41±1.31	67.75****±1.36	3.34±0.54
	S (48)	64.71±1.52	68.51****±1.60	3.80±0.45
Mean corpuscular hemoglobin(pg)	C (53)	20.92±0.52	20.92±0.52	0.00±0.13
	S (48)	21.01±0.66	20.73±0.63	-0.28±0.13
Mean cell hemoglobin Concentration (g/dl)	C (53)	32.37±0.30	30.77****±0.25	-1.60±0.17
	S (48)	32.20±0.38	30.06****±0.36	-2.14±0.23
Retinol (mg/dl)	C (53)	31.25±2.02	29.44±1.60	-1.81±1.19
	S (48)	27.34±1.35	35.92****±1.18	8.58####±1.60
Retinol-Binding (µg/l) Protein	C (11)	21.77±2.98	19.40*±2.65	-2.37±0.91
	S (11)	16.79±0.81	17.23±0.79	0.44##±0.58
Prealbumin (mg/dl)	C (11)	14.09±0.98	12.73*±0.92	-1.36±0.51
	S (11)	13.64±0.75	12.92±0.53	-0.72±0.61
Albumin (g/dl)	C (53)	4.85±0.05	4.82±0.05	-0.03±0.06
	S (48)	4.90±0.06	5.00*±0.03	0.10±0.05
Iron (µg/dl)	C (53)	44.42±2.89	49.10*±2.73	4.68±1.97
	S (48)	42.30±3.63	51.48±4.21	9.18±2.98
Ferritin (ng/ml)	C (53)	12.00±1.78	11.70±1.68	-0.30±0.50
	S (48)	10.73±1.76	10.43±1.58	-0.30±0.61
Transferrin (g/l)	C (11)	3.26±0.13	3.26±0.17	0.00±0.15
	S (11)	3.47±0.19	3.56±0.19	0.09±0.07
%saturation of transferrin	C (11)	12.82±1.74	12.73±1.54	-0.09±1.18
	S (11)	9.09±2.73	10.18±2.04	1.09±2.72

+C= Control group; S= Supplemented group
* Significantly different from baseline (*P<0.05; **** p<0.0001).
# Significantly different from control group (#p <0.05; ##P<0.01; #### p<0.0001).

Table VI is a comparison of the hematologic and biochemical variables in the control and supplemented groups at baseline and 6 weeks after vitamin A supplementation. It shows that at 6 weeks Hematocrit, Retinol and Retinol-bind¬ing protein had increased significantly in the supplemented albumin level.' group when compared to the control group. However, there was no significant difference between the two groups in any other variable.

Discussion

The cross-sectional analysis showed that 18 anemic, 4-8 year old children, were also suffenngfrom inadequate level of vitamin A (<20 ug/dl), out of which 2% were grossly deficient (<10 ug/dl). This is considered to be a public health problem accordingto the Pan-Arnerican Health Organizationcnteria[21]. A similar study carried out by Ibrahim et al[22] showed that 18% Karachi children under 15 years of age, had inadequate level of vitamin A.
Retinol was also found to be significantly though weakly correlated with Mean Corpuscular

Hemoglobin Concentration (MCHC), serum iron and ferritin. The multiple regression analysis further showed that, when adjusted for difference in age, sex and albumin level, Retinol was significantly positively associated with Hemoglobin and Hematocrit. This indicates that vitaminA and iron metabolism are inter-related. Three hypotheses have been put forward to explain the relationship observed between vitamin A and hematopoiesis.

1) Vitamin A influences the differentiation of the red cells[13], and/or 2) Vitamin A deficiency inhibits the mobilization of the endothelial iron deposits[23] and/or 3) Vitamin A deficiency increases one's susceptibility to infections and consequently to an impaired hematopoiesis[9]. However, this cross-sectional studies cannot prove a causative relation between vitamin A deficiency and anemia since an inadequate intake of both iron and vitamin A could have influenced the found association. Thus, to find if this association was causal, vitamin A supplementation trial was carried out.

It was observed that6 weeks aftcrvitamin Asupplementation, the hematocnt had improved significantly besides the serum Retinol and RBP levels in comparison with the control group. Since the dietaiy iron remained the same during this period, so probably the vitamin A supplement helped to raise the Hematocrit. Douer and Koeffler[13] showed that Retinoic acid (a metabolite of vitamin A) enhanced the growth of human early erytbroid progenitor cells in vitro. They suggested that Retinoic acid, in addition to its known effect on epithelial cells, may be involved in the growth of normal hematopoietic cells. This accounts for the significant increase in hematocrit. However, although significant, the increase is not great. Probably the time duration of the study (6 weeks) and the administration of a single dose of vitamin A, prevented any further increase in the hematocnt. Bloemetal" also failed to find a statistically significant difference in the erythrocyte indices 4 months after giving the supplement, but in a short term (2 weeks) study Bloem et al[12] found these parameters significantly elevated. So, with a single oral massive dose of vitamin A, after an initial spurt of activity in the bone marrow its effect gradually tapers down. Thus WHO[24] recommends periodic 3-6 monthly supplementation with vitamin A. Their chief aim is to reduce childhood morbidity and mortality but may have the added advantage of improving childhood anemia also. But long-term studies with repeated periodic vitamin A supplementation are necessaiy to further confirm this advantage.

In this study, no otherparameter of iron metabolism was significantly elevated e.g. Hemoglobin or MCH. Bloem et al[11] also failed to find a statistically significant difference in the hemoglobm level orMCH of Thai children,.2 or4 months after giving the vitarninA supplement. These findings contrast with the work of Hodges et al[25] who showed that experimental vitamin A deficiency in 8 middle aged American men was very significantly associated with a decrease of hemoglobin which returned to normal as soon as vitamin A was repleted. This apparent difference in the response of hemoglobin to vitamin A therapy may be explained by the initial difference in the iron status of the subjects. Pakistani and Thai children, who had low iron stores, as seen by their Ferntin levels, could not respond to the vitamin A stimulus by increasing the synthesis of Hemoglobin, but merely by increasing the red cell production as shown by an increase of hematocrit. In contrast, in the American study, the volunteers had normal hemoglobin and hematocrit levels at the startof the study. Artificial vitamin A deficiency, merely lowered the capacity for synthesis of Hemoglobin as shown by a drop in the hemoglobin level. But since the iron stores were normal, as soon as the vitamin A level was corrected, the hemoglobin synthesis returned to normal as seen by a normal Hemoglobin level. Thus-giving both iron and vitamin A may achieve a quicker cure of anemia than giving either nutrient seçpamtely. This was also shown in the work of Mejia and Chew[9].

The study thus concluded that the WHO recommended regimen of Periodic massive doses of vitamin A for children, to reduce morbidity and mortality, may have the added benefit improving their anemia as well. But longer studies with repeated vitamin A supplementation, are needed to arnve at this definite conclusion.

References

1. Food and Agriculture Organization, availability and consumption levels of iron and vitamin A. Rome. Food policy and nutrition division, Document No. 13, 1985.
2. Mejia, L.A., Hodges, RE., Arroyave,G. etal. VitaminA deficiency and anemia in Central American Children 1.2. Am. J. Clin. Nutr., 1977;30: 1175-84.
3. Mohanram, M., Kulkami, K.A. and Reddy, V. Hematological studies in vitamin A deficient children. Int.. J. Vitamin Nutr. Res., 1977;47:389-93.
4. Mejia, L.A. and Arroyave, G, The effect ofvitamin A fortification of sugar on iron metabolism in preschool children in Guatemala 1-3. Am. J. Clin. Nutr., 1982;36:87-93.
4. Mejia, L.A. and Arroyave, G. Lack of direct association between serum transferrin and serum biochemical indicators of vitamin A nutriture. Acts. Vitaminol. Enzymol., 1983;5:179-84.
6. Mejia, L.A. Vitamin A deficiency as afactor in nutritional anemia. Int. J. Vitam. Nutr. Res. (Suppl), 1985;27:75-84.
7. Araujo, R.L., Araujo, MB.D.G.. Machado. R,D.P. et al. Evaluation ofa program to overcome Vitamin A and iron deficiencies in areas of poverty in Minas Gerais. Brazil. Arch. Latinoam. Nutr., 1987;37:9-22.
8. Mejia, L.A. and Chew, F. Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. Am. J. Clin. Nutr, 1988;48:595-600.
9. Muhilal, H., Murdiana, A.. Aziz, I. et al. Vitamin A fortified monosodium glutamate and vitamin A status: A controlled field trial. Am. J. Clin. Nutr., 1988;48: 1265-70.
10. Bloem, MW., Wede!, M., Egger, RJ. et al. Iron metabolism and vitamin A deficiency in children in Northeast Thailand. Am. J. Clin. Nutr. 1989;50:332-8.
11. Bloem, M.W., Wedel, M., Agtmaal, E.J.V. eta!. Vitamin A intervention: short term effects of a single, oral, massive dose on iron metabolism. Am. J. Clin. Nutr., 1990;51:76-9.
12. Douer, D. and Koefiler, H.P. Retinoic acid enhances growth of human early erythroid progenitor cells in vitro. J. Clin. Invest., 1982;69:1039-41.
13. Micronutrient survey of Pakistan 1976-1977, Nutrition cell of planning and development division, Islamabad, Government of Pakistan, 1978.
14. National Nutrition Survey 1985-87 report Nutrition Division, National Institute of Health, Islamabad, Government of Pakistan, 1988.
15. Nutrition Survey of West Pakistan 1965-1966. the Directorate of Nutrition Survey and Research, Ministry of Health, Labour and Family Planning Islamabad, (Health Division), Government of Pakistan, 1967.
16. Bieri, J.G., Toltiver, T.J, and Catignani, G.L. Simultaneous determination of a.tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am. J. Clin, Nutr,, 1979;32:2143-9.
17. Mancini, G., Carbonara, AD. and Heremans, J.F. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 1965;2:235-54.
18. Whitehead, T.P., Thorpe, G.H.G., Carter, TiN. et al. Enhanced luminescence procedure for sensitive determination of peroxidase- labelled conjugates in immunoassay. Nature, 1983;305 :158-9.
19. Olsen, A.D. and Hamlin, W.B. A new method for serum iron and total iron binding capacity by atomic absorption spectrometry. Clin. Chem., 1969; 15:438-44.
20. Doumas, B.T., Watson, w. and Biggs, HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim Acts., 1971;31:87-96.
21. Pan American Health Organization (PAHO). Hypovitaminosis A in the Americas Report of a PAHO Technical Group Meeting. Sci. Publication No. 198, Washington, World Health Organization, 1970,
22. Ibrahim, K., Yousufi, MA., Hasnain, SN. et at. Plasma vitamin A and Carotene levels in Karachi

population. J. Pak. Med. Assoc., 1987;37: 117-26.
23. Staab, D.B., Hodges, R.E.,Metcalf, WK. et al. Relationship betweenvitamin A and iron in the liver. J. Nutr., 1984;114:840- 4.
24. World Health Organization. Vitamin A supplements: A guide to their use in the treatment and prevention ofvitamin A deficiency and xerophthalmia. Geneva, Prepared by WHO/UNICEF/NACG Task Force, 1988.
25. Hodges, RE., Sauberlich, HE., Conham, J.E. et a!. Hematopoietic studies in vitaminAdeficiency. Am. 3. Clin. Nutr., 1978;31;876- 85.