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ROLE OF MICRONUTRIENT MIXTURE IN ACUTE AND PERSISTENT DIARRHEA IN INFANTS AND ITS IMPACT ON NUTRITIONAL STATUS

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The present research dealt with comparing the effect of zinc-vitamin A-micronutrient mixture (ZAMM) administration on the nutritional status of infants with acute and persistent diarrhea. In addition, its effect on the management of both types of diarrhea was studied. Zinc and vitamin A were given as 8 mg and 751 μg retinol equivalent respectively per day.

The results showed that administration of ZAMM produced an increase in body weight and a significant increase in haemoglobin concentration in acute and persistent diarrheal cases with a significant increase in mean corpuscular volume in persistent diarrhea cases. Plasma zinc and vitamin A levels showed a significant increase in acute diarrheal infants after supplementation of ZAMM. Certain parameters, reflecting management of diarrhea such as appetite, stool frequency and duration of diarrhea, have been improved after ZAMM supplementation.

Conclusion: ZAMM administration improved both nutritional status and diarrheal state in infants with either acute or persistent diarrhea, however its effect was more promissing in acute cases.

INTRODUCTION

Diarrhea is among the most important causes of death among children in developing countries. In children, diarrhea is commonly classified into acute watery diarrhea and persistent diarrhea. Persistent diarrhea accounts for most diarrhea-associated deaths [Fauveau *et al.*, 1991]. However, the number of children dying each year from diarrhea has decreased over the past decade due to the success of oral rehydration solution [Claeson & Merson, 1990]. Although improved, the persistently high mortality rate evokes the need of additional strategies including micronutrients' supplementation. Therefore interventions that reduce diarrhea would be expected to have a large impact on child mortality. Zinc deficiency can cause impaired immune function and growth retardation both of which are risk factors for diarrheal illnesses [Allen, 1994; Beisel, 1982]. Malnutrition has been shown to be one of the important risk factors for diarrhea [Baqui *et al.*, 1993]. The benefits of zinc supplementation have not been universally observed in all children having diarrhea with malnutrition [Fuchs, 1998]. However some studies reported that zinc therapy shows promise in the treatment of diarrhea [Fuchs, 1998]. So several questions such as the optimal dose that should be given, optimal duration of therapy and the interaction of zinc and copper absorption have arrisen. Some studies show certain benefits of using zinc dose twice and 4 times the recommended dietary allowance [Fuchs, 1998]. However excessive amounts of zinc can also result in treatment failure, and perhaps worse, depressed fungicidal activity consistent with impaired monocyte function as well as increased incidence of impetigo in infants [Schlesinger *et al.*, 1993]. The optimal duration of therapy has yet to be defined. Zinc supplementation has the potential to aggravate marginal copper deficiency at a dose of 18.5 mg/day only 3.5 mg more than RDA in young men [Fosmire, 1990].

Children with acute diarrhea in association with malnutrition require supplementation with not only zinc but with other micronutrients as well. So a micronutrient mixture containing zinc would be more beneficial than zinc alone for diarrheal infants [Cousins & Hempe, 1990]. Other micronutrients such as vitamin A and folate have been reported to have an important impact in management of diarrheal illness [Gracey, 1999].

The present study dealt with malnourished infants with either persistent or acute watery diarrhea, supplemented with zinc-vitamin A-micronutrient mixture (ZAMM). Zinc and vitamin A were given as 8 mg and 2500 IU (751 μg retinol equivalent), respectively, per day for only 10 days so as to study the beneficial effect of this dose level and time of supplementation.

The aim of the present search was to compare the effect of ZAMM intervention in infants with acute watery and persistent diarrhea. The comparison was based on its impact on diarrheal management, plasma zinc and vitamin A levels and the nutritional status.

SUBJECTS, MATERIALS AND METHODS

SUBJECTS

The study has been carried out in Al-Sahel Teaching Hospital, Cairo Egypt. The study dealt with 20 infants with

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acute and persistent diarrhea attending the nutrition clinic, their age ranged from 6.5 to 24 months; and ten healthy control infants, with matched age and sex. The diarrheal infants were 14 with acute diarrhea and 6 with persistent diarrhea. Infants with diarrhea of duration more than 14 days were considered as persistent diarrheal infants [Sazawal *et al.*, 1996].

MATERIALS

Zinc-vitamin A-micronutrient mixture, under the commercial name of vitam syrup, produced by Pharco Pharmaceuticals, contained in each 10 mL: 2500 IU of vitamin A (equal to 751 μg retinol equivalent), 8 mg zinc from zinc sulfate, 400 IU (equivalent to 10 μg cholecalciferol) of vitamin D_3, 10 mg vitamin E, 40 mg vitamin C, 0.7 mg vitamin B_1, 0.8 mg vitamin B_2, 0.7 mg vitamin B_6, 3 mcg vitamin B_{12}, 9 mg nicotinamide, 5 mg calcium pantothenate, 200 mcg folic acid, 150 mcg biotin, 10 mg iron from ferrous sulfate.

METHODS

After being properly hydrated, all diarrheal infants were weighed. Their mid arm circumferences (MAC) were measured. Nutritional status of infants was deduced from NCHS (National Center for Health Statistics, USA) (<70 % of standard were considered severely malnourished, 70–80% moderate, 80–90% mild, and > 90% normal).

Blood samples were taken from fasted infants for determination of blood picture, including leucocytic count, lymphocytes, RBCs, platelets, blood haemoglobin concentration [Betke & Savelsberg, 1956] and haematocrit percentage [Strumia *et al.*, 1954] with the calculation of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) according to Dacie and Lewis [1984]. Plasma was separated for determination of plasma albumin [Doumas *et al.*, 1972], zinc [Homsher & Zak, 1985], copper [Abe *et al.*, 1989], iron [Tabacco *et al.*, 1981], and total iron binding capacity [Tietz, 1982]. The percentage of transferrin saturation was calculated from plasma iron and total iron binding capacity. Plasma vitamin A was determined by HPLC [Janebova & Zima, 1997]. The conditions of HPLC were as follows: column: sepharon $SDXC_{18}$ 4×250 mm, 7 μm particle size (TESSEK,CZ), loop 20 μL, mobile phase 100% methanol; flow rate: 1 mL/min; detection: 325 nm, UV detector LCD 2082; HPLC equipment (ECOM, CZ).

Biochemical data of patients were compared with those of control using statistical analysis of Student's *t*-test (2 – tailed).

Each diarrheal infant was given 10 mL of ZAMM per day (in two divided doses) for 10 days. Mothers were advised not to give their infants ZAMM during breast feeding or shortly after or before breast feeding or meals, to avoid dietary inhibitors of zinc absorption such as phytate and casein and at the same time to avoid the inhibiting activity of zinc towards absorption of dietary copper. So it is preferred to administer ZAMM apart from rather than during a meal or breast feeding.

It is worth mentioning that the recommended dietary allowances of vitamin A, vitamin D, vitamin E, vitamin C, vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, nicotinamide, folic acid, biotin, zinc, iron, calcium and pantothenic acid for 0.5–1 year old and 1–2 years old infants are: 375 and 400 μg retinol equivalent, 10 and 10 μg cholecalciferol, 4 and 6 mg α-tocopherol, 35 and 40 mg, 0.4 and 0.7 mg, 0.5 and 0.8 mg, 0.6 and 1 mg, 0.5 and 0.7 μg, 6 and 9 mg niacin equivalent, 35 and 50 μg, 15 and 20 μg, 5 and 10 mg, 10 and 10 mg, 600 and 800 mg, 3 and 3 mg, respectively [National Research Council, 1989].

Blood samples were taken from diarrheal infants at the end of the clinical study for redetermination of the fore--mentioned biochemical parameters. In addition, remeasu-

TABLE 1. Blood picture and different biochemical parameters of acute (n = 14) and persistent (n = 6) diarrheal infants before and after ZAMM administration in comparison to control subjects (n= 10) (values are mean SE).

Parameters	Control	Acute		Persistent	
Blood picture:		Before ZAMMA	After ZAMM	Before ZAMM	After ZAMM
Hematocrit [%]	31±1.2	28±1.1	31.6±3.6	23.0±3.8	29.1±4.1
Haemoglobin [g/dL]	10.7±0.4	9.6±0.3^{a}	10.2±1.2	7.7±1.2^{a}	10.6±2.3
RBCs/L	$3.8{\pm}0.2 \times 10^{12}$	$3.7{\pm}0.2 \times 10^{12}$	$3.9{\pm}0.5 \times 10^{12}$	$3.3{\pm}0.6 \times 10^{12}$	$3.6{\pm}0.7 \times 10^{12}$
MCV [fl]	81.5±1.7	75.7±1.5^{b}	81.7±5.1	69.7±1.7^{e}	80.8±2.3^{G}
MCH [pg]	28.2±0.6	25.9±0.5^{c}	26.2±1.7	23.3±0.6^{e}	29.4±0.61
MCHC [g/L]	345±3.5	343±3.3	323±11.5	335±10.2	364±11.3
WBCs/L	$10.6{\pm}1.0 \times 10^{9}$	$10.5{\pm}0.9 \times 10^{9}$	$10.8{\pm}3.8 \times 10^{9}$	$8.8{\pm}2.2 \times 10^{9}$	$8.7{\pm}3.1 \times 10^{9}$
Lymphocytes/L	$5.04{\pm}0.16 \times 10^{9}$	$3.81{\pm}0.21 \times 10^{9e}$	$4.5{\pm}0.5 \times 10^{9}$	$4.9{\pm}0.42 \times 10^{9}$	$5.3{\pm}0.3 \times 10^{9}$
Platelets/L	$271{\pm}26.4 \times 10^{9}$	$231{\pm}14 \times 10^{9}$	$207{\pm}36 \times 10^{9}$	$263{\pm}27.4 \times 10^{9}$	$260{\pm}30 \times 10^{9}$
Plasma:					
Albumin [g/dL]	3.37±0.21	3.29±0.25	3.59±0.26	2.9±0.27	3.28±0.34
Zinc [μg/dL]	87.8±6.4	67.4±6.2^{a}	97.7±7.3^{G}	68.2±6.03^{a}	75.4±13.34
Copper [μg/dL]	96.6±8.1	90.5±7.8	94.3±9.2	105±10.57	40.6±13.44
Iron [μg/dL]	75.1±14.0	71.9±7.9	84±8.6	76±10.8	84.3±26.15
TIBC [μg/dL]	313±21	310±30	309±26	-	-
Transferrin saturation [%]	24±7.9	23.2±8.0	27.2±6.1	-	-
Vitamin A [μg/dL]	31.0±3.1	22.7±2.5^{a}	33.3±4.5^{F}	30.4±4.6	30.85±8.92

Values significantly differ from control: a – $p < 0.05$; b – $p < 0.025$; c – $p < 0.010$; d – $p < 0.005$; e – $p < 0.001$. Values significantly different when data after supplementation were compared with those before supplementation: F – $p < 0.05$; G – $p < 0.005$.

rements of body weights were carried out. Other parameters that reflect diarrheal management such as stool frequency, consistency, duration of diarrhea and appetite improvements were followed before and during ZAMM administration. Data before and after ZAMM administration were compared using statistical analysis of Student's *t*-test.

Questionnaire was done to know the number of breast--fed and artificially-fed infants.

RESULTS

Comparison of the base line features of diarrheal infants with control healthy infants (Table 1) showed significantly decreased haemoglobin concentration, MCV, MCH and plasma zinc in both acute and persistent diarrheal infants. Lymphocytic count and plasma vitamin A showed significantly reduced level in infants with acute diarrhea compared with control. No significant differences have been noticed in other determined parameters, including plasma iron. However, remarkable low levels in plasma albumin, haematocrit, and WBCs count were noticed in infants with persistent diarrhea. Notably low levels of percentage haematocrit and plasma copper were observed in infants with acute diarrhea.

Administration of ZAMM (Table 1) produced insignificant but remarkable increase in haemoglobin concentration in both acute and persistent diarrhea with significant increase in MCV in the case of persistent diarrhea ($p<0.005$). Plasma zinc level and vitamin A showed significant increase on supplementation of ZAMM in the case of infants with acute diarrhea ($p<0.005$, 45% and $p<0.05$, 47%, respectively). A remarkable but insignificant increase (11%) in plasma zinc was noticed in infants with persistent diarrhea on supplementation of ZAMM. Lymphocytic count showed notable but not significant increase in infants with acute and persistent diarrhea after administration of ZAMM.

Studying the nutritional status of the base line through anthropometric parameters (Tables 2 and 3) showed 4 infants with moderate malnutrition, one with mild malnutrition and one was normal from infants with persistent diarrhea according to both weight/age and mid arm circumference/age. However, concerning infants with acute diarrhea, 2 were severely malnourished, 8 were mildly malnourished, and 4 were normal according to weight/age, but according to mid arm circumference/age, 2 were moderately and 12 were mildly malnourished. After administration of ZAMM there were increases in mean body weights in acute and persistent diarrheal infants (+130g and + 50 g, respectively) (Table 4).

TABLE 2. Nutritional status of infants with persistent diarrhea according to both weight/age and mid arm circumference/age (values are mean±SD).

Parameters	Nutritional status		
	Moderate malnutrition	Mild malnutrition	Normal
Age [months]	9±1.2	24	6
Weight [kg]	6.75±0.29	11	8.5
MAC [cm]	11.7±0.23	14.4	14.6
No	4	1	1

TABLE 3. Nutritional status of infants with acute diarrhea according to weight/age and MAC/age (values are mean±SD).

I – According to weight/age:

Parameters	Nutritional status		
	Severe malnutrition	Mild malnutrition	Normal
Age [months]	24±0	17.5±3.4	10.5±1.1
Weight [kg]	8.5±0.1	9.54±0.7	8.5±0.6
No	2	8	4

II – According to MAC/age:

Parameters	Nutritional status	
	Moderate malnutrition	Mild malnutrition
Age [months]	9±0	17.7±4.4
MAC [cm]	12±0.1	13.6±0.4
No	2	12

TABLE 4. Body weight of infants with acute and persistent diarrhea before and after ZAMM administration (values are mean±SD).

Parameters	Type of diarrhea			
	Acute		Persistent	
	Before	After	Before	After
Age [months]	16.43±5.14	–	11±6.5	–
Weight [kg]	9.09±0.81	9.22±0.78	7.75±1.75	7.8±1.5

Mean stool frequency in infants with acute diarrhea was 8.3±0.437 times/day and was reduced to 2.6±0.137 after 4.1±0.312 days of ZAMM administration. While in those with persistent diarrhea, it was 8.7±0.494 times/day and was reduced to 3±0.447 after 6.2±0.939 days of ZAMM supplementation. This means that duration of diarrhea in the acute cases was significantly ($p<0.05$) lower than in the persistent cases after ZAMM supplementation. The stool consistency of the infants with acute diarrhea was watery in 12 cases and loose in 2 cases, and changed after 4.1±0.312 days to well formed in 12 cases and to 2 semiformed. In the infants with persistent diarrhea, all cases were watery and changed after 6.2±0.939 days to 4 well formed and 2 semiformed.

Appetite was improved after 3.3±0.125 and 6.0±0.821 days of ZAMM administration in acute and persistent diarrheal cases, respectively. Appetite was significantly improved ($p< 0.005$) in the acute diarrheal cases compared to the persistent cases after ZAMM administration.

All infants with persistent diarrhea were breast-fed. Ten cases of infants with acute diarrhea were breast-fed and 4 were artificially-fed.

DISCUSSION

Base line feature of cases with diarrhea in the present study showed 5 cases from 6 of the infants with persistent diarrhea and 10–14 cases from 14 cases of those with acute diarrhea were malnourished with reduced serum zinc level in both persistent and acute cases. It has been reported that zinc deficiency is associated with subnormal growth, decreased appetite and abnormalities in the senses of taste and smell [Ney, 1984] which may have relation to anorexia and weight loss that often accompanied diarrhea [Fuchs, 1998]. Nutritional status is arguably the most critical host factor that determines risk and expression of diarrheal morbidity and mortality [Pelletier, 1995]. Indeed, diarrheal disease and malnutrition coexist in children from developing world and the most consequential childhood diarrheal disease in terms of mortality is associated with malnutrition and zinc deficiency [Fuchs, 1998].

In a previous study [Soliman *et al.*, in press] with diarrheal children of age ranging from 3 months to 5 years, we have observed similar results concerning base line where plasma zinc levels and blood haemoglobin concentration were reduced significantly in both acute and chronic cases with reduction of vitamin A only in acute cases. Also there were no significant changes in plasma copper and iron. However in the previously-mentioned study, zinc reduction was more pronounced in chronic cases than acute which is not shown in the present study. This might be due to the fact that children were of older age than in the present study, so they were exposed to more recurrent diarrheal episodes which may lead to such reduction. In the present study 100% of persistent diarrheal cases were breast-fed in comparison to 70 % of acute cases which may result in less zinc deficiency in persistent cases. It has been reported that extensive breast feeding may result in lower zinc deficiency in addition to preventing and controling diarrhea [Sazawal, 1996; Gracey, 1999].

Cellular immunity is associated with a higher incidence of acute and persistent diarrhea [Baqui *et al.*, 1993]. The influence of zinc deficiency on cellular immune responses is well documented. Negative zinc balance [Castillo-Duran *et al.*, 1988] and depressed plasma or tissue zinc concentration during diarrhea in children [Naveh *et al.*, 1982] have been observed. Zinc deficiency has been reported to reduce intestinal immune clearance mechanisms for pathogens. Zinc deficiency also induced reversible ultrastructural changes in villous architecture and Paneth cells, gut permeability and disaccharidases which can affect duration of diarrheal episode. Zinc depletion is associated with reduction in lymphocytic count, T-helper cell proportion and produced lymphoid tissue atrophy. All of these impairments respond dramatically to zinc repletion [Sazawal, 1996]. Vitamin A deficiency also depresses helper T lymphocyte activity which may lead to severe infection such as diarrhea [Carmen *et al.*, 1989]. In the present study, lymphocytic counts along with plasma levels of zinc and vitamin A were significantly lower in the infants with acute diarrhea than control healthy infants. It has been reported that deficiency of zinc and vitamin A may be the risk factors for diarrhea and that supplementation of both could prevent the incidence of diarrhea [Tomkins, 1991; Perez *et al.*, 1994].

Although small increase in body weight was observed on supplementation of ZAMM in both persistent and acute diarrheal infants, however it reflected the successful use of ZAMM in promoting growth during diarrheal episodes, in the infants with acute diarrhea body weight gain was 130 g while in those with persistent diarrhea it was 50 g after 10 days of ZAMM administration. In previous studies [Roy *et al.*, 1997; 1998], supplement of zinc for 14 days produced body weight gain of 120 g in acute diarrheal children and maintained body weight in persistent diarrheal children, however diarrhea without any supplementation induced body weight reduction by 80 g in persistent cases and an increase by 30 g in acute ones. Zinc has been reported to increase intestinal absorption [Rodriguez *et al.*, 1996] thereby may increase body weight.

The present study also showed that ZAMM has an important role in management of diarrhea as manifested by stool frequency, consistency, duration of diarrhea and rapid improvement of appetite. However its role is more pronounced in acute cases compared with persistent cases as shown from duration of diarrhea which was 4.1 ± 0.3 days and 6.2 ± 0.9 days respectively, and from improvement of appetite which was after 3.3 ± 0.1 days and 6.0 ± 0.8, respectively. Stool frequency was reduced to ≤ 3 times/day indicating recovery according to Sazawal *et al.* [1996]. Black [1998] reported efficacy of zinc in promoting recovery from acute watery diarrhea and diminution of diarrhea associated growth failure. Zinc supplementation during acute and persistent diarrhea in children has been reported to reduce diarrheal episode duration and severity [Sazawal *et al.*, 1995] either through an improvement in the immune status or by reversing the changes that occur in the gut due to zinc deficiency. Zinc supplementation has been reported to improve appetite, acceleration of mucosal regeneration, enhance cellular immunity and lead to enhanced catch-up growth *via* stimulating insulin-like growth factor I [Folwaczny, 1977]. Roy *et al.* [1997; 1998] noticed an increase in serum zinc in the zinc-supplemented group (+2.4, +0.2 μmol/L) versus a decrease in unsupplemented group (-0.3 and –1.8 μmol/L) in acute and chronic diarrheal children, respectively. The increase in serum zinc and lymphocytic count after supplementation of ZAMM reflected improvement of nutritional status of zinc and immune function that have direct relationship to diarrheal management.

Copper absorption has been reported to be impaired by zinc administration which is very important in malnourished children in whom copper status might already be marginal [Cousins & Hempe, 1990]. The importance of copper is due to its essential role to maintain normal immune function [Castillo-Duran *et al.*, 1988]. In the present study, the base line of patients showed serum copper level slightly reduced only in persistent diarrheal infants compared to healthy control, however in acute diarrheal infants, serum copper was slightly higher. These results showed no association between serum copper level and malnutrition, since the majority of cases under study were malnourished. Supplementation of ZAMM did not show any significant change in copper level except slight reduction in the infants with persistent diarrhea. This may be due to precausions given to mothers in order not to give their infants ZAMM during meals or may be due to the low dose of supplemented zinc (8 mg/day) compared to other studies.

Base line of iron status of the infants with diarrhea though showed normal levels of plasma iron, however significantly reduced haemoglobin, MCV and MCH reflected anaemic state of both types of diarrhea. Previously, Roy *et al.* [1997] reported reduced blood haemoglobin during both persistent and acute diarrhea. ZAMM supplementation produced some improvements in iron status reflected by insignificant increase in haemoglobin, haematocrit and MCH in both types of diarrhea and a significant increase in MCV and insignificant increase in plasma iron in persistent diarrhea. This might be due to the presence of iron in the supplemented mixture in addition to vitamin C that promotes iron absorption. Vitamin B_{12} and folate may also help to improve iron status because they are necessary for erythrocyte formation [Mayes, 1996].

The reduction of plasma vitamin A in infants with acute diarrhea showed complete improvements after ZAMM, though the supplementation was only for 10 days. Not only vitamin A in ZAMM that may induce increase in plasma vitamin A but also zinc because it is necessary for a normal plasma vitamin A concentration due to its key role in the synthesis of retinol – binding protein [Smolin & Grosvenor, 1997]. Diarrhea over 4 days has been reported to significantly reduce serum vitamin A [Agarwal *et al.*, 1996], so the increased plasma vitamin A during ZAMM in acute diarrheal cases in the present study is considered a successful result.

The supplementation of zinc-multi–micronutrients mixture in diarrheal infants with malnutrition may be more preferred than giving zinc alone. This is because they are needed for improving the nutritional status with consequent improvement of diarrheal state. The presence of antioxidants such as vitamin E, A and C in ZAMM is important since recent study [Ashour *et al.*, 1999] showed reduced antioxidant state in protein-malnourished children. The presence of biotin, B-vitamins, vitamin D, vitamin E, vitamin A and vitamin C in ZAMM may lead to improvement of immune function [Blumberg, 1994; Mayes, 1996] thereby may improve diarrheal state.

CONCLUSION

The administration of 8 mg zinc/day with the micronutrient mixture used in the present study in the malnourished diarrheal infants of age from 6.5 to 24 months for 10 days has beneficial effect. The beneficial effect is represented by the improvement of nutritional status, which was demonstrated by an increase in the body weight, plasma zinc and plasma vitamin A and by management of diarrheal state. However its effect was more promissing in acute than persistent diarrhea cases.

REFERENCES

1. Abe A., Yamashita S., Noma A., Sensitive direct colorimetric assay for copper in serum. Clin. Chem., 1989, 552, 35.
2. Agarwal D.K., Singh S.V., Gupta V., Agarwal K.N., Vitamin A status in early childhood diarrhea, respiratory infection and in maternal and cord blood. J. Trop. Pediatr., 1996, 42, 12–4.
3. Allen L.H., Nutritional influences on linear growth: a general review. Eur. J. Clin. Nutr., 1994, 48, (Suppl. I), S 75–S 89.
4. Ashour M.N., Salem S.I., El–Gad ban H.M., Elwan N.M., Basu T.K., Antioxidant status in children with protein energy malnutrition living in Cairo, Egypt. Eur. J. Clin. Nutr., 1999, 53, 669.
5. Baqui A.H., Sack R.B., Black R.E., Chowdhury H. R., Yunus M. Siddique A.K., Cell-mediated immuno--deficiency and malnutrition are independent risk factors for persistent diarrhea in Bangladeshi children. Am. J. Clin. Nutr., 1993, 58, 543–548.
6. Beisel W.R., Single nutrients and immunity. Am. J. Clin. Nutr., 1982, 35, 417–468.
7. Betke K., Savelsberg W., Styfenphoto-matrisho haemoglobin estmmung mittels cyan – haemoglobin. Z-Biochem., 1956, 320, 431.
8. Black R.E., Persistent diarrhea in children of developing countries. Pediatr. Infect. Dis. J., 1993, 129, 751–761.
9. Black R.E., Therapeutic and preventive effects of zinc on serious childhood infectious diseases in developing countries. Am. J. Clin. Nutr., 1998, 68 (suppl.), 476 S–9 S.
10. Blumberg J.B., Nutrient control of immune function, 1994, *In*: Functional Foods. Designer Food, Pharma Foods, Nutraceuticals. (ed. Goldberg), New York, Chapman Hull, Inc., p. 87.
11. Carmen J.A., Smith S.M., Hayes C.E., Characterization of a helper T lymphocyte defect in vitamin A deficient mice. J. Immunol., 1989, 142, 388.
12. Castillo–Duran C., Vail P., Uovy R., Trace mineral balance during acute diarrhea in infants. J. Pediatr., 1988, 113, 452–457.
13. Claeson M., Merson M.H., Global progress in the control of diarrheal diseases. Pediatr. Infect. DiS. J., 1990, 9, 345–55.
14. Cousins R. J., Hempe J.M., 1990, Zinc in Present Knowledge in Nutrition, (ed. M. L. Brown), 6th ed., Washington, DC: International Life Sciences Institute, Nutrition Foundation, 251, 60.
15. Dacie S.J.V., Lewis S.M., 1984, Practical Hematology. Sixth edition Churchill Livingstone, Edinburgh London Melbourne and New York, p. 22.
16. Doumas B. T., Waston W.A., Biggs H. G., Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta., 1972, 31, 87.
17. Fauveau V., Yunus M., Zaman K., Chakraborty J., Sarder A.M., Diarrhea mortality in rural Bangladeshi children. J. Trop. Pediatr., 1991, 37, 31–36.
18. Folwaczny C., Zinc and diarrhea in infants. J. Trace Elem. Med. Biol., 1997, 11, 116–22.
19. Fosmire G.J., Zinc toxicity. Am. J. Clin. Nutr., 1990, 51, 225–227.
20. Fuchs G.J., Possibilities for zinc in the treatment of acute diarrhea. Am. J. Clin. Nutr., 1998, 68 (suppl), 480 S–3 S.
21. Gracey M., Nutritional effects and management of diarrhea in infancy. Acta Pediatr. Suppl., 1999, 88, 110–26.
22. Homsher R., Zak B., Spectrophotometric investigation of sensitivity complexing agents for the determination of zinc in serum. Clin. Chem., 1985, 31, 1310–1313.
23. Janebova M., Zima T., Methods for determination of vitamins A and E, our simple HPLC assay. Sborn. Lek., 1997, 98, 195–208.
24. Mayes P.A., Structure and function of the water–soluble vitamins, 1996, *In*: Harper's biochemistry. (eds. R. Murray, D. Granner, P.A. Mayes, Rodwell), 24th ed., a LANGE medical book, p. 599.
25. National Research Council. 1989, Recommended dietary allowances. 10th ed., Washington, DC; National Academy Press.
26. Naveh Y., Lightman A., Zinder O., Effect of diarrhea on serum zinc concentrations in infants and children. J. Pediatr., 1982, 101, 730–733.
27. Ney D., Nutritional assessment, 1984, *In*: Manual of Pediatric Nutrition. Printed in USA, (eds. D.G. Kelts E.G. Jones), 1st edition, Little, Brown and Company.

Boston/Toronto, p. 99.
28. Pelletier D.L., Potentiating effects of malnutrition on child mortality: epidemiologic evidence and policy implications. Food Nutr. Bull., 1995, 16, 206–13.
29. Perez F.A.M., Sagaro G.E., Fragasa A.T., Marrercy R.R., Serum levels of vitamin A and their relation with acute and persistent diarrhea. Acta Gastroenterol. Latinoam., 1994, 24, 255–60.
30. Rodriguez P., Darman N., Chappuis P. Candalh C., Blaton M.A., Bouchoud C., Heyman M., Intestinal paracellular permeability during malnutrition in guinea pigs: effect of high dietary zinc. Gut, 1996, 39, 416–22.
31. Roy S.K., Tomkins A. M., Mahalansbis O., Akramuzzaman S.M., Haider R., Behrens R.H., Fuchs G., Impact of zinc supplementation on persistent diarrhea in malnourished Bangladeshi children. Acta Paediatr., 1998, 87, 235–9.
32. Roy S.K., Tomkins A.M., Akromuzaman S.M., Behrens R.H., Haider R., Mohalanafisd D., Fuchs G., Randomized controlled trial of zinc supplementation in malnourished Bangladeshi children with acute diarrhea. Arch. Dis. Child., 1997, 77, 196–200.
33. Sazawal S., Black R.E., Bhan M.K., Bhandari N., Sinha A., Jalla S., Effect of zinc supplementation during acute diarrhea on duration and severity of the episode, a community based double blind controlled trial. N. Engl. J. Med., 1995, 333, 839–844.
34. Sazawal S., Black R.E., Bhan M.K., Jalla S., Bhandari N., Sinha A., Makimdar S., Zinc supplementation reduces the incidence of persistent diarrhea and dysentery among low socioeconomic children in India. J. Nutr., 1996, 126, 443–450.
35. Schlesinger L., Arevalo M., Arredondo S., Lonnerdal B., Stekel A., Zinc supplementation impairs monocyte function. Acta Paediatr., 1993, 82, 734–8.
36. Smolin A.L., Grosvenor B.M., 1997, Nutrition Science and Applications, 2nd edition Sounders Collage Publishing, USA, p. 484.
37. Soliman S.M., Abou Sekkien M., Hamed Th., EL–Behairy H.F., El–Keiy M., Al–Okbi S.Y., Acute and chronic diarrhea in children: Dietary management and its impact on nutritional status. Medical J. Cairo Univ. Accepted in January, 2001 (in press).
38. Strumia M.M., Sample A.B., Hart E.D., An improved micro–haematocrit method. Am. J. Clinical Pathology, 1954, 221, 1016.
39. Tabacco A., Moda E. Tarli P., An improved, highly sensitive method for the determination of serum iron using chromazurol B. Clin. Chim. Acta, 1981, 114, 287–290.
40. Tietz N.W., 1982, In Fundamentals of Clinical Chemistry. NW. Tietz, Editor, Saunders, Philadelphia, USA, pp. 924–929.
41. Tomkins A., Recent developments in the nutritional management of diarrhea. 1. Nutritional strategies to prevent diarrhea in children in developing countries. Trans. R. Soc. Trop. Med. Hyg., 1991, 85, 4–7.

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