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PROTOZOAL INFECTIONS IN ZINC DEFICIENT CHILDREN FROM RURAL AREAS AROUND MANSOURA CITY

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ABSTRACT

Zinc deficiency in children is an important public health problem in the developing world (Manary et al, 2002). Several lines of evidence suggest that zinc status of our infants and children are marginal or low. First, animal products, the main source of zinc, represent only a small percentage of the usual diet. Second. high consumption of rice and vegetables may preclude adequate zinc absorption because of their high phytate and fiber contents. Third, gastrointestinal disease (diarrhea, parasites) may increase intestinal losses of zinc. So, in our locality, the magnitude of marginal zinc deficiency problem in apparently healthy infants and children is expected to be high (Hegazi et al., 2002).

The pattern and intensity of protozoal infections in zinc deficient children were studied in comparison to children with normal serum zinc level. The present study (case control study) was conducted on 55 children from rural areas around Mansoura. attending the outpatient clinic of Mansoura University Children's Hospital. They were of both sexes and their ages ranged from 4-11 years. Cryptosporidium parvum was the commonest parasite prevalent among both groups. Other protozoal infections detected were Entamoeba histolytica, Giardia lamblia, with a prevaof 60.6% and 57.6% respectively in children with low serum zinc compared to 54.5%, and 50% in children with normal serum zinc level. These differences were

statistically insignificant. However, zinc deficient children had statistically significant heavy intensity of protozoal infections compared to children with normal serum zinc level. Also, it was observed that zinc deficient children showed statistically significant co-infection with 3 protozoa compared to children with normal serum zinc level who suffered from only one or two protozoal infection at most.

INTRODUCTION

Zinc deficiency contributes to growth retardation and impaired cognitive development in children moreover it is found to be associated with an increased prevalence of infections (Shankar, 2000). Several micronutrients such as vitamin A, beta carotene, folic acid, vitamin B12, vitamin C, iron, zinc and selenium have immono-modulating functions and thus influence the susceptibility of a host to infectious disease (Bhaskaram, 2002).

Parasitic infections of the gut are highly prevalent in developing countries and contribute to the several malnutritions associated with persistent diarrhea in children (Sullivan et al., 1992).

All infectious agents, Particularly protozoa and helminth parasites act as very complex antigens and elicit a range of immune responses, a proportion of which are relevant to protection. By the beginning of the 1990s a general pattern emerged in which it appeared that, in the majority of protozoal infections in experimental modeles. Th1 cells were protective and Th2 were counter protective (Cox and Wakelin, 1998), where as in helminthic infections the reverse was the case. Th2 cells were protective and Th1 cells were counter-protective (MacDonald et al., 2002).

Deficiencies in zinc are highly linked to the immune system, causing rapid loss in cell and antibodymediated response. This deficiency increase the susceptibility to a variety of pathogens that place the children in many developing countries at increased risk of illness and death from infectious disease (Black, 2003).

Both humoral and cell mediated immunity are suppressed by zinc defi-

ciency (Beisel, 1988), but it appears that cell-mediated immunity may be more sensitive (Harrer et al., 1992). Importantly, T-cell mediated responses are critical for host protection against parasitic infections.

According to Prasad (1998), zinc deficiency caused an imbalance between Th1 and Th2 function. The production of INF-y, IL-2 and TNF-γ (products of TH1) cells were decreased, whereas the production of IL-4, IL-5, IL-6, and IL-10 (products of Th2) were not affected during zinc deficiency.

Serum zinc levels in-patients with hookworm, Enterobius vermicularis infection were significantly lower than unifected controls (Migasena et al., 1984; Koltas et al., 1997).

Karakas et al., (2001) reported tat there was a significant decrease in serum zinc levels in children with amebiasis and giardiasis when compared to the control in whom no infestation with intestinal parasite. They also found that after metronidazole therapy, a significant increase in serum zinc levels of patients compared

to their control.

The zinc status of the body can be measured in a variety of ways, especially by determination of the zinc content of blood plasma or serum, blood cells (red or white), saliva, sweat, skin, nails, hair and urine (Gutbrie and Piccian, 1995). Measurement of serum zinc concentration is the usual method of assessing zinc status (Baynes and Dominiczak, 1999).

The aim of this work was to determine the prevalence and intensity of protozoal infections in zinc deficient children compared to children with normal serum zinc level.

MATERIAL AND METHODS

The patients groups comprised 55 children of the same socioeconomic level from rural areas around Mansoura, the capital of Dakhlia governorate. They were attending the outpatient of clinic of mansoura University Pediatric Center. Their ages ranged from 4 to 11 years. Thirty four were males and twenty one were females. They were divided according to their weight for age, which is considered

as the most common nutritional index used in children and provides a good genral expression of growth status as documented by Trowbridge (1988) into:

A) Malnourished (under-weight) groups (25): B) Well-nourished (normal weight for age) group (30).

Underweight children due to chronic illnesses as congenital discase, endocrinal, renal, hepatic, and neoplastic disorders as well as children with short stature were excluded from the study. All children were subjected to the following:

- A thorough history taking with special concern to gastrointestinal symptoms as diarrhea, anorexia, recurrent abdominal pains, weight loss, flatulence and passage of blood and mucous with stools.
- Anthropometric measurements as weight for age, height for age, head circumference for age, and weight for height percentile.
- Complete general and systemic examination.
- 4) Urine and stool examination.
 - Examination of urine for eggs using sedimentation

method.

b. Examination of stool by direct smear, formal-ether concentration technique, (Harada-mori culture), zinc sulphate centrifugal floatation method, special stains for diagnosis of intestinal protoza (Trichrome staining technique and modified Zeil-Neelsen staining).

Intensity of infection was estimated by Katothick smear (Marten and Beaver 1968). Counting the number of oocvsts in ten high power field (H.P.F.) (Addiss 1991) and the standard method for counting the number of cysts in the stool (Danciger and Lopez, 1975). All stool samples were examined fresh within three hours after collection if there is a delay of more than 3 hours after collection the stool was refrigerated at 4 c for 24 hours.

 Assessment of serum zinc level using colorimetric method (Spinreact. Espania) (Homsher and zak, 1985).

RESULTS

Table (1): Prevelance of protozoal infections in zinc deficient children versus children with

Parasitic infection	Children with zinc deficiency (33)		Children with normal serum zinc level (22)		P	Well nourished children (30)		Mal nourished children (25)		P
	N	%	N	%		N	%	N	%	
Entaemeba histolytica	+ve 20	60.6	+ve 12	54.5	0.5	+ve 18	60.0	+vc 11	44.0	0.8
Giardia Iamblia	+ve 19	57.6	+ve 11	50.0	0.6	+ve 16	53.3	+ve	64.0	0.4
Crypto- sporidium	+ve 23	69.7	+ve 13	59.1	0.06	+ve 17	56.6	+vel5	60.0	0.3

Table (2): Comparison between well nourished and malnourished children:

Point of study	Well nouris	hed children	Malnourished (25)	P	
	Mean SD	Range	Mean SD	Range	
Age	5.86 1.88	4.9-11	5.07 1.58	4.5-10.6	0.16
Weight	21.82 18	15-32. 7	16.71 3.49	12.2-20.3	0.02*
Height	113.64 29	98.9-137	110.07 12.06	95.8-130	0.45
Diarrhea (number of +ve cases and percentage)	5 (16.66%)		10 (40.0%)	0.04*	
Serum zinc (ug/dl)	92.64 25.50	85.6-145	60.09 0.12	33.2-72.1	0.03*
Intensity of Entaemeba histolytica infection Cyst count/mg stool	38.03 11.42	10-120	139.59 23.53	150-650	0.01*
Intensity of Giardia lamblia infection Cyst count/mg stool	39.95 14.2	5-105	171.45 46.32	50-520	0.001**
Intensity of Cryptosporidium parvum infection Oocyst/H.P.F.	3 0.3	5-10	16 5	20-200	0.001**

Table (3): Comparison between zinc deficient children and children with normal serum zinc level:

Point of study	Children wi deficiency		Children with serum zinc le	P		
	Mean SD	Range	Mean SD	Range		
Age	60.04± 1.14	4.2-9.6	6.57±1.65	4.9-11	0.08	
Weight	16.94±5.13	15.54-29.1	19.87±4.33	16.87-32.7	0.05*	
Height	109.42±12.32	105.2-134	113.11±12.14	111.2-137	0.06	
Diamhea (number of +ve cases and percentage)	23 (69.7%)		7 (31.8%)		0.02*	
Serum zinc (ug/dl)	55.51±7.86	33.5-69	87.05±21.92	91-145	0.03*	
Intensity of Entaemeba histolytica infection Cyst count mg stool	187.25±108.23	50-700	56.6±21.04	5-150	0.003**	
Intensity of Giardia lamblia infection Cyst count/mg stool	250.53±114.31	70-630	64.103±38.5	33-104	0.001**	
Intensity of Cryptosporidium parvum infection Oocyst/H.P.F	29±11	10-150	6±2	4-20	0.001**	

Table (4): Co-infection with 3 protozoa in zinc deficient children versus children with normal zinc level, and in well nourished children verses malnourished children.

	Children with zinc deficiency (33)		Childr with serum level (2	normal zinc	P	Well nourished children (30)		Malnourish ed children (25)		P
	N	%	N	%	0.01	N	%	N	%	
Co fection with 3 protzoa	-ve 23	69.7% 30.3%	+ve 5	22.73% 77.27%	*	+ve 6	20% 80.0%	+ve 16	64.0%	0.01*

Table (5): Correlation between intensity of protozoal infection & serum zinc level, and weight for height percentage:

		Entaemeba histolytica cyst count/mg stool		nblia Cyst ig stool	Cryptosporidium Oocyst/HPF	
	Kendall's tau-b	P	Kendall's tau-b	P	Kendall's tau-b	P
Serum zin level	085	0.54	-0.079	0.53	-0.08	0.49
Weight heigh percentile	t -0.07	0.59	-1.0	0.001**	-0.085	0.51

DISCUSSION

Trace mineral deficiencies may affect several biological functions in human and thus influence the susceptibility of a host to infectious disease (Bhaskaram, 2002).

This study revealed that serum zinc levels below normal in 60% (33 out of 55 children) while in 40.0% levels were within norma I range table (3). This relatively high prevalence of zinc deficiency in our children is close to that of other children in developing countries. Based on serum or plasma levels, around 30-50% of children residing in low income settings have low serum or plasma zinc. Mild to moderate zinc deficiency is common in these countries because of a low dietary intake of zinc-rich animal-source foods in which zinc is more bioavaliable, high consumption of cereal grains and legumes, which contain inhibitors of zinc absorption and an overall poor dietary intake. Children in these countries are also frequently affected by enteric infection, which result in excess fecal losses of zinc (Bhatnagar, and Natchu. 2004).

In this study, prevalences of helminthic infections such as Ascaris lumbricoides. Enterobius vermicularis, and Strongyloides strecoralis, as well as, Hymenolepis nana infection were extremely low and statistically insignificant. On the other hand, a high prevalence of protozoal infection was evident. Entamoeba hisolytica and Giardia lamblia showed a prevalence of 60.6% and 57.6% respectively in children with low serum zinc compared to 54.5%, and 50.0% in children with normal serum zinc level. Prevalences of Entamoeba hisolytica and Giardia lamblia in malnourished children were 44.0%, and 64.0% respectively compared to 60.0%, and 53.3% in well nourished children. These result were close to the regional prevalence of amebiasis and giardiasis in the tropics (Weissman and Salata, 2000& Pickering, 2000).

The most striking for attention was the high prevalence of Cryptosporidium parvum, with reported prevalence of 69.7%, 59.1, 60.0% and 58.6% in zinc deficient children, children with normal serum zinc level, malnourished children, and well nourished children respectively. This is a rela-

tively high prevalence of Cryptosporidium parvum compared to a prevalence rate of 31.6% in Bolivian children, which was attributed to the poor sanitation conditions, contaminated water supplies, overcrowding, and close contact with domestic animals (Esteban et al., 1998). This high prevalence of Cryptosporidium parvum is usually encountered in outbreaks caused by contamination of filtered public water supplies (Hayes et al., 1989, Joseph et al., 1991, and Mac Kenzie et al., 1994).

Although, Cryptosporidiosis is a self-limiting disease in immunocompetent hosts, and chronic diarrhea is common in individuals with immunodeficiency, such as congenital hypogammaglobulinemia or HIV infection (Flynn, 2000), this high prevalence of Cryptosporidium parvum in our study may be an indirect evidence and a serious alarm of contamination of our local water supplies.

The pattern of protozoal infections was similar between zinc deficient children and their controls as well as between malnourished and well nourished children, most probably a re-

flection of the same environmental influence and a similar socioeconomic level. Regarding the intensity of infection. Our study revealed a highly significant increase in E.histolytica, Giarlamblia cvst count. Cryptosporidium oocyst (table:3), and significantly higher rates of coinfection with 3 protozoas (table:4) in zinc deficient children compared to children with normal serum zinc level. This could be explained by lowering of cell mediated immunity which is sensitive to zinc deficiency (Harrer et al., 1992). Importantly, T-cell mediated response are critical for host protection against parasitic infections (Mitchell, 1980), Premature transition from efficient Th1-dependent cellular immune function to Th2-dependent humoral immune function leading to shift in Th1-depending cellular immune function to Th2-dependent humoral immune function leading to shift in Th1/Th2 balance towards Th2 may be related to deficiencies of zinc. nitrogen monoxide, and /or glutathione (Sprietsma, 1999). Thus, the impact of zinc status on host immune functions are the intensity, duration, and the severity of parasitic infection (Scott and Koski, 2002).

Also, a simlar statistically significant increase in the intensity of protozoal infection (table:2) and rates of co-infection with 3 protozoas (table:4) in malnourished children in comparison to well nourished children, was detected. So, the increased parasitic intensity in malnourished children. may be affected mainly by nutritional status rather than zinc deficiency alone. This may agree with the experimental study done by Boulay et al., (1998), he and his colleague concluded that low dietary protein but not low zinc increased host susceptibility to Heligmosomoides polygyrus reinfection and increased worm burden and egg ouput in challenge infection. However, it is worth to mention thata significantly lower serum zinc level in malnourished childre in relation to well nourished children was evident in our study and should be considered. and may emphasis the role of zinc in protection against protozoal infection. It is also known that, malnourished children are often reported to have associated micronutrient deficiencies (Khanum etal., 1988). Micronutriet deficiencies and infections disease often coexist and exhibit complex interactions lading to the vicious cycle of

malnutrition and infections among underprivileged populations of the developing countries particularly preschool children (Bhaskaram, 2002).

Regarding the clinical data of studied children, a significantly higher prevalence of diarrhea in zinc deficient children compared to children with normal serum zinc level, and in malnourished children compared to well nourished children, was found. Diarrhea is a frequent symptom of parasitic infection of the intestine. Absorptive defects for one or more nutrient may accompany excessive fluid and electrolyte loss (Broitman and Zamcheck, 1980). Parasitic microorganisms initiate bi-directional interactions with the defense mechanisms of the host, both immunological and cellular, and also interact with nutritional status of the host (Beisel, 1984). In a study conducted by Oberhelman et al., 1998, parasitosis was more prevalent among children under age 2 years with low height for age, while low weight for age was more closely associated with parasitic infection in order children. There are many studies emphasizing the possible explanations for the link between zinc defi-

ciency and diarrhea, functional impairment of the immune system and of intestinal mucosal cell transport mechanisms are suggested (Ghishan, 1984). On the other hand, people known to be infected with intestinal parasites, which cause bleeding into the bowel, or malabsorption can cause losses of zinc or failure of absorption (Check et al., 1981). In addition to the losses of zinc in diarrheal fluid, during the diarrhea the compensatory mechanisms are less effective for absorption of divalent elements such as zinc, which remain bound to ligands of dietary or endogenous origin (Wapnir, 2000). Finally, zinc deficiency could induce uroguanylin levels in the intestine and cause or potentiate diarrhea. Uroguanylin, a natriuretic peptide hormone, is an endogenous ligand for the same guanylate cyclase C that the Escherichia coli heat-stable toxin binds when it causes secretory diarrhea (Blanchard and Cousins, 2000).

No significant correlation between intensity of protozoal infection and serum zinc level or weight for height percentile was documented, which copes with the findings of Muniz

(2002), who sated that no significant relationship between intestinal parasitic infection and children's weight was detected, and concluded that a small but significant negative relationship between intestinal helminthic infection and children's growth was detected in his study in an urban environment with low prevalence of both intestinal parasitic infection and malnutrition. However, a highly nificant negative correlation between intensity of Giardia lamblia infection and weight for height percentile was evident, which may be related to the important role of Giardia in chronic diarrhea, malabsorption, and failure to thrive or weight loss (Pickering, 2000).

CONCLUSIONS

This study showed a high prevalence of protozoal infections especially Cryptosporidium parvum which approach prevalence of cryptosporidiosis outbreaks caused by contaminated water supplies (Hayes et al., 1989, Joseph, et al., 1991, and Mac Kenzie etal., 1994). This may a serious alarm for poor quality of our water supplies, and deserves our extreme attention to prevent contamina-

tion of our water supplies as well as following strict measures to improve sanitary conditions. Boiled water may be used in certain circumstances as most of protozoa are heat sensitive but chlorine resistant (Weissman and Salata, 2000 & Pickering, 2000).

No significant differences in the patterns or prevalences of protozoal infections between zinc deficient children and children with normal serum zinc level were detected in this study but a significant increase in the intensity of protozoal infection and co-infection with 3 protozoa in zinc deficient children was clearly evident. Thus, the impact of zinc status on host immune function are the intensity, duration, and severity of parasitic infections (Scott and Koshi, 2000).

Measurement of serum zinc concentration is the usual method of assessing zinc status (Baynes and Dominiczak, 1999). Serum zinc is influenced by growth velocity, catabolism, and infections, confounding interpretation, however, serum zinc is regarded as a reasonable indicator of zinc status at the group level (Michaelsen etal., 1994). T-cell mediated response are critical for host protection against parasitic infection (Mitchell, 1980), and cell mediated immunity may be more sensitive to zinc deficiency than humoral immunity (Harrer ea al., 1992). Intensity of protozoal infections may be affected by the nutritional status as a whole and not only by serum zinc alone, however, serum zinc is an integral part of nutritional status and serum zinc was also, lower in malnourished than well nourished children in our study.

Zinc deficiency is common in our children with high prevalence approaching 60%, so the magnitude of zinc deficiency problem is high in our locality, and active interventions to prevent zinc deficiency should be undertaken.

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الملخص العربى

يمثل نقص مستوى الزنك مشكلة صحية هائلة في الدول النامية وقد أظهرت دراسات عديدة أن مستوى الزنك في الأطفال المصريين قليل حيث أن الغذاء الحيواني هو المصدر الرئيسي بينما يمثل الغذاء الحيواني نسبة قليلة في غذائنا بالإضافة الى إستهلاك كميات من الغذاء النباتي يحتوى على الأليات التي تقلل إمتصاص الزنك ويضاف الى ذلك إنتشار النزلات المعوية بين الأطفال وقد أجريت هذه الدراسة لمعرفة العلاقة بين نوع البروتوزوا وشدة الإصابة في الأطفال الذين ينخفض فيهم مستوى الزنك.

تضمنت الدراسة ٥٥ طفل من الريف حول مدينة المنصورة يأتون للكشف في مستشفى الأطفال الجامعي بمدينة المنصورة وقد قسم الأطفال الى مجموعتين لتجانس في السن والعمر تبعاً لمستوى النمو (الوزن)، أجرى لهم فحص البراز وقياس مستوى الزنك وأظهرت النتائج أن البرتوزرا كريتوسبروديم هو الأكثر إنتشاراً في المجموعتين ولايوجد فرق معنوى بين المجموعتين بالنسبة لنوع البروتوزوا وقد أظهرت الدراسة أن مستوى الزنك منخفض وذو دلالة معنوية خاصة في الأطفال الذين يعانون من إصابة شديدة كماً وكيفاً.