Review

Intestinal Parasitic Infections Including Cryptosporidiosis And Immunological Aspects Among Malnourished Children

Raghad M Jassim, M.Sc.*
Nadham K. Mahdi, M.Sc., Ph.D.*
Mea'ad K. Hassan, C.A.B.P.**

الملخص

أجريت هذه الدراسة الاستقصائية على ٣٩٤طفل (٢١٧ ذكر و١٧٧ انثى)دون سن الخامسه من العمر. كان ١٩٤ طفل منهم كانوا مصابون بسوء التغذية اخذوا من مستشفى البصرة للولاده والطفل وقد تبين ان (٨٤٪) منهم يعانون من الهزال ، (٨,٢٤٪)الهزال مع نقص الوزن و(٦,١٨٪) نقص الوزن .

تكون الاصابات الطفيلية اكثرانتشارا" بين الاطفال الذين يعانون من سوء التغذية (٥٩,٨٪) مقارنة مع الاصحاء (٣٣٪) وتزداد هذه النسبة بشكل واضح لدى الاطفال الذين يعانون من سوء التغذيه المصحوب بالاسهال (٣٢,٥٪).

وجد داء خبيات الابواغ بنسبة (٦,٨٥٪) بين الاطفال دون سن الخامسة من العمر وكانت اعلى نسبة(١٤,٨٩٪) لدى الاطفال المصابين بسوء التغذية ويعانون من الاسهال مقارنة مع الاطفال المصابين بسوء التغذية ولايعانون من الاسهال حيث كانت (١١٪),كما وجدت الاصابة بنسبة (٢٪) لدى الاطفال الاصحاء ويعانون من الاسهال ولم تسجل اى حالة اصابة بين الاطفال الاصحاء الذين لايعانون من الاسهال.

سجلت المناعة الخلوية انخفاضا" معنويا في العدد الكلي للخلايا اللمفية وبشكل خاص الخلايا اللمفية التائية عند الاطفال المصابين بسوء التغذية سواء كانوا يعانون من الاسهال ام ل ابينما المناعة الخلطية المتمثلة بالامينوكلوبيلينات من نوع أي وام وجي فقد سجلت ارتفاعا"عند الاطفال المصابين بسوء التغذية سواء كانوا يعانون من الاسهال ام لامقارنة مع الاطفال الاصحاء وبوجود فارق احصائي معتد.

وقد سجل المتمم ارتفاعا" لدى الاطفال المصابين بسوء التغذية ولايعانون من الاسهال مقارنة بالاصحاء وبوجود فارق احصائي معتد في المتمم من نوع (٤) وعدم وجود فارق احصائي معتد في المتمم (٣) وفي حالة الاسهال فقد لوحظ انخفاضه عند الاطفال المصابين بسوء التغذية والذين يعانون من الاسهال وبوجود فارق احصائي معتد في المتمم من النوع (٣). اما فيما يخص عملية البلعمة فقد سجلت انخفاضا" عند الاطفال المصابين بسوء التغذية سواء كانوا يعانون من الاسهال او لا وبوجود فارق احصائي معتد.

Abstract

This is a case-control study, which involved 194 malnourished children below 5 years of age who were attending Basrah Maternity and Children Hospital during 2001. About 84% have marasmus, 8.24% marasmun-kwashiorkor, 6.18% kwashiorkor and 1.55% under weight.

Parasitic infections were more frequent among malnourished children (59.8%) than wellnourished children (33%), with increased frequency among malnourished children with diarrhea (32.5%). Cryptosporidiosis was found in 6.85% of children below 5 years with increased frequency among malnourished children with diarrhea 14.89% compared to 11% in malnourished children without diarrhea. Only 2% were noticed among wellnourished children with diarrhea while no case has been recorded in wellnourished children without diarrhea.

Depressed cellular immunity characterised by decrease in total lymphocytes (particularly T-lymphocytes) was found among malnourished children either with or without diarrhea. While humoral immunity (IgG,

* Department of Microbiology,
** Department of Pediatrics,
College of Medicine, University of Basrah, Basrah, Iraq.

IgM, IgA) was significantly elevated among malnourished children with or without diarrhea in comparison to wellnourished children. C3 and C4 were increased in malnourished children without diarrhea with significant difference in C4 only. While they were decreased in malnourished children with diarrhea, with significant difference in C3. In addition, Phagocytic activity showed a significant decrease in malnourished children with or without diarrhea compared to wellnourished children.

Key words: Children, Cryptosporidiosis, Diarrhea, Immunity, Malnutrition, Parasitic infections.

Introduction

Malnutrition is a major contributor to child morbidity and mortality [1]. It may be due to improper or inadequate food intake and absorption, insufficient food supply and poor dietary habits [1]. In addition to the consequent impairment of physical growth and physiologic function, immune response changes would occur. These immune response changes are predisposing children to opportunistic and other typical childhood infections.

One of the important caused of malnutrition is diarrhea. This is because patients with diarrhea eat less and their ability to absorb nutrients is reduced. Moreover, their nutrient requirements are increased as a result of infection[2]. Malnutrition occurring after repeated episodes of diarrhea can make diarrhea more severe, prolonged, frequent and have a higher case fatality rate compared with diarrhea in wellnourished children [3,4].

Malnutrition is the most frequent cause of secondary immunodeficiency worldwide. It damages almost all layers of the protective umbrella of immunity including cell replication and protein synthesis [5]. However, the most severe effects appear to be related to cell mediated immunity rather than humoral immunity [6,7]. This mean that there are a reduction in the numbers of T-lymphocytes while the numbers of B-lymphocytes remain either normal, or rarely may be decreased or increased [8-10]. In malnourished children, the ingestion of particles by phagocytic cells is intact but the opsonization is reduced at low plasma level [8,9,11]. Also, there is a reduction in the serum level of almost all complement proteins [11].

Therefore, malnutrition generally alters resistance of the host to parasitic infection, and infectious disease aggravates the existing malnutrition. So, this study is designed to investigate the interaction of malnutrition, intestinal parasitic infections including cryptosporidiosis and immunological status in children with or without diarrhea.

Materials and Methods

Patients: Stool samples were collected from 194 malnourished children (104 boys and 90 girls) during 2001. Their ages ranged from 3months to 5years. Ninety-four of them had diarrhoea that has been admitted to the pediatric wards of Maternity and Children Hospital in Basrah for treatment. The rest were without diarrhoea and were attending the rehabilitation center. Further 100 wellnourished children (63 boys and 37 girls) with diarrhoea who were either in or outpatients were included as a control group. Also, 100 wellnourished apparently healthy children (50 boys and 50 girls) who were not complaining of diarrhoea for the last 2 weeks.

Anthropometric measurements:

Weight, height, weight for height for age and weight for age were recorded for each child (patients and control). They were estimated according to CDC, WHO, Nation Centre for Health Science normalised references [3,12,13]. Weight was determined by an infant weighing scale and the child was freed from heavy clothing before weighing. Recumbent length was measured for children who couldn't stand using rollameter by Raven, while the standing height was measured for children who could stand, without shoes, on a portable measuring board to the nearest 1 cm.

Stool examination: Direct smear method and then formalin-ether sedimentation concentration method [14] were carried out for all stool samples to detect ova, cysts and trophozoites of intestinal parasites. Fecal smears were prepared from the sediment and stained by the modified Ziehl-Neelsen method[14] for the

recovery of acid-fast oocysts of *Cryptosporidium.*

Immunological study: One and half ml blood were taken and kept in plain test tube. Sera were separated by cntrifugation at 3000 r.p.m. for 5min and used for estimation of IgG, IgM, IgA and complements C3 and C4[15] (Biomagherb). Four ml of blood were obtained from 50 children (patients and control) and mixed with heparin for detection of E-Rosette. Further one ml of blood was collected into EDTA tube for detection of phagocytic activity by using chemiluminesence assay [16]. The principle of this assay is the oxidation

Table- 1. Distribution of malnourished children in relation to different anthropometric (nutritional) indices.

Nutritional status		Weight/Age No. (%)	Weight/ Height No. (%)	Weight/ Age No (%)
Malnutrition with diarrhea	≤-1SD	0 (0.0)	19 (20.21)	50 (53.19)
	-2SD	5 (5.32)	20 (21.28)	6 (6.38)
	-3SD	42 (44,68)	30 (31.91)	24 (25.54)
	-4SD	47 (50.0)	25 (26.61)	14 (14.89)
Malnutrition without diarrhea	-1SD	3 (30.0)	36 (36.0)	22 (22.0)
	-2SD	29 (29.0)	34 (34.0)	17 (17.0)
	-3SD	46 (46.0)	23 (23.0)	26 (26.0)
	-4SD	22 (22.0)	7 (7.0)	15 (15.0)

Well nourished children usually above -1SD.

of luminol by reactive radical oxygen produced during phagocytosis in phagocytes; luminol enhances the amount of light generated by polymorph nuclear leukocyte upon stimulation with the soluble barium sulphate. The reaction mixture consisted of 2 ml of assay inducer, 0.2 ml Nacl (Oxoid), 0.2 ml of luminol (Sigma) in a 5 ml beaker, to this mixture 0.02 ml of whole blood was added and agitated to mix well. This mixture is then placed into the measuring cuvette of photon counting system, chemiluminescence was continously recorded on a chart recorder, inform of definite curve. The measurement is repeated twice to demonstrate the influenced variables.

Also, total and differential counts of white blood cells were done for each sample.

Statistical analysis: Standard Normal Deviate (SND) was used to assess the significance of difference between groups. P-value less than 0.05 was considered as statistically significant.

Results

This is a case-control study, in which mild and moderate forms of (low weight for height) malnutrition represent about 36% and 34% respectively, in malnourished children without diarrhea and in malnourished children with diarrhea, they represent 20.21% and 21.28%. While severe malnutrition was present in 58.56% of malnourished children with diarrhea compared to 30% in malnourished children without diarrhea (Table -1).

According to the Wellcome classification of malnutrition, which depends on the clinical features, marasmus was the most frequent type of malnutrition 84.02%, followed by marasmus-kwashiorkor 8.24% and kwashiorkor 6.18% .

Malnourished children with diarrhea had the highest percent (14.89%), followed by malnourished without diarrhea (11%) and wellnourished with diarrhea (2%). No case had been recorded in wellnourished children without diarrhea (Table 2). The higher rate of parasitic infections including cryptosporidiosis was found in malnourished children with diarrhea (67.02%) ($P<0.01$) followed by malnourished children without diarrhea (53%) ($P<0.01$), wellnourished children with diarrhea (48%) ($P<0.01$) while the lowest rate was observed in wellnourished children without diarrhea (18%) ($P<0.01$) (Table 2). The distribution of *Cryptosporidium* in relation to the severity of malnutrition had been illustrated in (Table -3).

Among the symptomatic cryptosporidial infections, watery diarrhea was more common (68.75%) than bloody diarrhea (31.25%), weight loss (100%) followed by fever (81.25%), vomiting (62.5%) and cough in (31.25%).

A decrease in total lymphocytes (particularly T-lymphocytes) was found among malnourished children (either with or without diarrhea) with significant difference, while IgG, IgM, IgA levels were significantly elevated among malnourished children (either with or without diarrhea) in comparison to wellnourished children (Table -4). Complement C3 and C4 were increased in malnourished children without diarrhea with significant difference in C4

and insignificant in C3; while they were decreased in malnourished children with diarrhea, with significant difference in C3. Phagocytosis showed a significant decrease in malnourished children (either with or without diarrhea) compared to wellnourished children (Table- 4).

Table- 2: Parasitic infections among children included in the study .

Parasites	MN.children with D. n=94 No. (%)	MN.children withouD. n=100 No (%)	WN.childrenwithD n=100 No (%)	WN.children without.D n=100 No (%)	Total 394
Cryptosporidium sp	11 (11.7)	7 (7)	-	-	8 (4.57)
Blastocystis hominis	12 (12.77)	12 (12)	13 (13)	4 (4)	44 (11.17)
Entamoeba histolytica	16 (17.02)	7 (7)	18 (18)	1 (1)	42 (10.66)
Giardia lamblia	19 (20.22)	15 (15)	11 (11)	9 (9)	58 (14.72)
Enterobius vermicularis	-	2 (2)	-	3 (3)	5 (1.26)
Cryptosporidium+E.histolutica	1 (1.06)	-	-	-	1 (0.25)
Cryptosporidium+G.lamblia	1 (1.06)	2 (2)	1 (1)	-	4 (1.02)
Cryptosporidium+B.hominis	1 (1.06)	2 (2)	1 (1)	-	4 (1.02)
E.histolytica+B.hominis	1 (1.06)	-	-	-	1 (0.25)
G.lamblia+B.hominis	1 (1.06)	2 (2)	-	1 (1)	4 (1.02)
G.lamblia+E.histolytica	-	1 (1)	-	-	1 (0.25)
Total parasitic infection	63 (67.02)	53 (53)	48 (48)	18 (18)	182 (46.19)
No pathogen	31 (32.98)	47 (47)	52 (52)	82 (82)	212 (53.81)

The comparison between:-	SND-test	P-value	
1-Malnourished and wellnourished in general MN=malnourished.	5.306	P<0.01	MN=malnourished.
2- Malnourished and wellnourished without diarrhea WN=wellnourished.	5.15	P<0.01	WN=Wellnourished.
3- Malnourished and wellnourished with diarrhea	2.714	P<0.01	D = Diarrhoea.
4- Malnourished with and without diarrhea	2.028	P<0.05	
5- Wellnourished with and without diarrhea	4.55	P<0.01	

Discussion

Malnutrition is a major factor leading to the high rates of childhood morbidity and mortality and to poor growth in children. If it is sufficiently severe, will impair resistance to infection [6] such as cryptosporidiosis and other intestinal parasitic infections.

Marasmus is the commonest form of malnutrition reported in this study, severe form of malnutrition like kwashiorkor, was more frequent among malnourished children with diarrhea rather than malnourished children without diarrhea. This is might be due to losses of nutrients and fluid with stool during diarrhea. Higher rate of parasitic infections including cryptosporidiosis was present in malnourished children than in wellnourished children (P<0.01) which might indicates that, malnutrition may be an important factor in the determining increases in the frequency and severity of infectious illnesses [8], since nutritional deficiency leads to a significant increased susceptibility to infection through depression of the immunological function as stated in this study and Dowd and Heatley [7], as well as changes in gastro-intestinal morphology and function [17].

This is the first study of cryptosporidiosis (14.89%) in association with malnutrition in Iraq, which was lower

Table-3: Distribution of Cryptosporidium positive cases in relation to the severity of malnutrition.

Weight/ Height	Malnutrition with diarrhea		Malnutrition without diarrhea	
	No examined	Cryptosporidium positive No. (%)	No. examined	Cryptosporidium positive No. (%)
-1SD	19	2(10.52)	36	7(19.44)
-2SD	20	3(15.0)	34	2(5.88)
-3SD	30	3(13.33)	23	1(3.34)
-4SD	25	5(20.0)	7	1(4.34)
Total	94	14(14.14.89)	100	11(11.0)

Table -4: Immunologic parameters of children involved in the study

Immunity		MN.with D.	MN.Without D.	WN.with D.	WN.without D.
Humoral immunity	IgG	N=36 R=294-2851 1617.86± 658.69	n=36 R=741-258 1426.72±462.77	n=30 R=870-2514 1473.467± 452.189	N=31 R=432-1609 938.64±255.25
	IgM	n=36 R=53-404 184.22±86.84	n=36 R=66-312 175.22±61.06	n=30 R=73-270 155.866±60.97	N=31 R=41-211 117.54±47.02
	IgA	n=36 R=44-415 203.05±87.25	n=36 R=65-400 181.63±73.22	n=30 R=79-363 174.06±70.01	N=31 R=38-189 97.516±34.373
Complement	C_3	n=12 R=37-259 137.25±73.83	n=12 R=95-259 180.5±63.46	N=13 R=163-266 198.61±28.403	n=13 R=152-321 219.307±51.06
	C_4	n=12 R=10.4-63.7 38.35±51.27	n=12 R=45.9-89.9 58.6±12.25	N=13 R=26.8-67.2 43.98±14.65	n=13 R=20.9-52.1 33.32±11.438
E-rosette		n=12 R=47-621 256.16±182.53	n=12 R=127-506 304.4±125.9	N=13 R=155-1996 1062.6±571.4	n=13 R=828-2142 1394.07±411.15
Total lymphocyte		n=12 R=585-7392 2820.41± 2250.87	n=12 R=910-6240 3131.25±1440	N=13 R=1296-8712 4574.3± 2327.53	n=13 R=4410-9792 6319.12±1954.79
Phagocytosis		n=12 R=0.002-0.009 0.01±0.015	n=12 R=0.0031-0.016 0.0066±0.0036	N=13 R=0.002-0.064 0.029±0.016	n=13 R=0.014-0.041 0.024±0.008

- Values were expressed as mean ± SD.
MN= malnutrition.
D = diarrhoea.
WN= wellnutrition.
R = range.

than that reported in Mexican (30.7%) [18] or West Indian (23.7%) [19] children.

Results of this study are supported by the fact that predominant symptoms of cryptosporidiosis in addition to diarrhea, are weight loss, vomiting and fever [21,22]. Cough had been recorded as well. However, various studies in different countries showed conflicting results. Even studies in the Mediterranean region are scar, but for instance, in Egypt, the predominant symptoms were vomiting and abdominal pain (100%) followed by urinary tract infection, anorexia and pyrexia (57%) [23]. While in infected Mexican children the main symptoms were vomiting (73%), followed by fever (69.2%), dehydration (38.4%) and bloody stool (23%) [17]. Therefore, a differential laboratory diagnosis is essential for proper treatment.

The response of each immunological variable to malnutrition has been variously reported to be depressed,. normal or elevated in different studies[8-10]. In this study, humoral immunity that includes IgG, IgM, IgA, tended to be higher in malnourished children with or without diarrhea. This is considered to be the result of repeated infection experienced by undernourished individuals, recurrent protracted infection of the gastro-intestinal and respiratory tract that occur partly as a result of impaired mucosal immunity [8]. A similar result had been reported by other studies [7,10,24].

In this study, the reduction in C3 level in malnourished children is associated with the change of protein metabolism and this may be due to reduction in its synthesis in the liver and increased breakdown [8,10].

This study also indicates that chemiluminescence assay is a simple, fast, cheap, reliable and accurate method for estimation of phagocytic activity. Decreased

phagocytizing activity of leukocytes in children suffering from malnutrition could be attributed to the lowering in glycolytic process during phagocytosis which supplies the necessary energy for particle uptake and low stimulation of hexose-monophosphate shunt activity which is the pathway for the direct oxidation of glucose and production of H_2O_2 and this component was responsible for bactericidal activity of leukocytes[16].

In conclusion there was a great interaction between malnutrition, parasitic infections including cryptosporidiosis and immunological status of the children in the region.

References

1- Curran JS, Barness LA. Nutrition. In: Bhrman RE, Kliegman RM, Jenson HB (eds). Nelson Textbook of Pediatrics, 16th ed. WB Saunders Co., Philadelphia 2000, pp. 138-88.

2- World Health Organization reports. Reading on diarrhea. Students Manual, Geneva 1992, pp. 3-14.

3- World Health Organization reports. Manual on malnutrition, management of child with a serious infection or severe malnutrition, Geneva 2000, pp. 80.

4- Briend A, Hassan K, Aziz KMA et al. Are diarrhea control programs likely to reduce childhood malnutrition; observation from rural Bangladesh. Lancet 1989; 5: 319-22.

5- Chandra R. Measuring impact using immunologic techniques. Nutr Rev 1981; 39: 225-31.

6- Scrimshaw NS, Sangiovanni JP. Synergism of nutrition, infection and immunity. Am J Clin Nutr 1997; 66: 464-77.

7- Dowd PS, Heatley RV. The influence of undernutrition on immunity. Clin Sci 1984; 66: 241-8.

8- Chandra RK. Interaction of nutrition, infection and immune response. Act Pediatr Scand 1979; 68: 137-44.

9- Kapoor OP. Immunity and vegetarianism. Am J Clin Nutr 1991; 35: 1-4.

10- Rickimaru T, Taniguchi R, Yartey JE et al. Humoral and cell-mediated immunity in malnourished children in Gana. European J Clin Nutr 1998; 52: 344-50.

11- Chandra RK, Kumari S. Symposium, Dietary nucleotides: A recently demonstrated requirement for cellular development and immune function. J Nutr 1993; 124: 1433-5.

12- World Health Organization report. Management of severe malnutrition. Geneva 1999, pp. 4-10.

13- Waterlow C. The presentation and use of height and weight data for comparing nutritional status of groups of children under the age of 10 years. Bull WHO 1977; 55: 489-98.

14- Markell EK, Voge M, John DT. Medical Parasitology. 6th ed. WB Saunders Co., Philadelphia. 1986, pp. 331.

15- Bauer JD, Ackermann PG, Toro G. Clinical Laboratory Methods. 8th ed. The C.V. Mosby Co., Saint Louis. 1974, pp. 50-1, 85-6.

16- Al-Hashimi AM, Mohammed FH, Lazim SKA. Comparative study of haemoglobin estimated by traditional WHO technique and cheminescence method. Dirasat 1997; 24: 161-77.

17- Sallon S, Deckelbaum RJ, Schmid II et al. *Cryptosporidium,* malnutrition and chronic diarrhea in children. Am J Comm Dis 1988; 142: 312-4.

18- Enriquez FJ, Avila CR, Santos JI et al. *Cryptosporidium* infection in Mexican children: nutritional, enteropathogenic and diagnostic evaluations. Am J Trop Med Hyg 1997; 56: 254-7.

19- Macfarlane DE, Horner BJ. Cryptosporidiosis in well-nourished and malnourished children. Acta Pediatr Scand 1997; 75: 474-7.

20- Mahdi NK, Al-Sadoon IO, Mohamed AT. First report of cryptosporidiosis among Iraqi children. Eastern Mediterranean Hlth J 1996; 2: 115-9.

21- Association of Medical Microbiologists. Facts about *Cryptosporidium.* 1997. (http: //www.amm.co.uk/pubs/fa_crypto.htm).

22- Khashba A, Hilali M, Marei M et al. Cryptosporidiosis among children suffering from diarrhea in Benha, Egypt. J Egyptian Soci Parasitol 1989; 19: 701-5.