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Fungal and Bacterial Infection in Malnourished Children and its Relation to Severity of the Disease

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The present research was conducted to assess the prevalence of fungal and bacterial infections in malnourished children and correlating these infections with the severity and type of malnutrition. The study included 50 malnourished children (25 Marasmus and 25 Kwashiorkor KW), their ages ranged from 1 to 36 months with a mean of 10 ± 7 months. All patients were subjected to full history taking, thorough clinical examination: Anthropometric measures: Routine investigations and Microbiological study (fungal and bacterial cultures) of urine samples obtained by suprapubic aspiration both rectal and mouth swabs as well as gastric aspirate using nasogastric feeding tube were taken from each patient. The present study revealed that *Candida albicans* was the most prevalent fungal pathogen isolated, constituted 81% of +ve fungal cultures (85% in marasmus and 15% in KW patients). *E. coli* was the most prevalent bacterial pathogen, detected in 50.5% of all \pm ve cultures (57% in marasmus and 43% in KW patients). The severity of childhood malnutrition determines the incidence of fungal and bacterial infection.

Key words: Fungal infection, bacterial infection, malnourished children, marasmus and Kwashiorkor (KW)

INTRODUCTION

Worldwide, malnutrition is one of the leading causes of morbidity and mortality in childhood (Churran and Barness, 2000). Undernutrition is the outcome of the interaction between poor diet and disease, it contributes to most of the growth deficits observed in children in less developed countries (Unicef, 1998).

Protein Energy Malnutrition (PEM) is the most common cause of acquired immunosuppression (Christie *et al.*, 1992; Bohler and Wathne, 2000). The decrease in both II-2 and II-4 may be due to dysregulation of T-lymphocyte generation cycle which leads to immune dysfunction in T-cell sub-populations. The decrease in both cytokines in PEM children leads to direct impairment in both T and B-cell maturation, differentiation and antibody production as well as on the inflammatory response and other related specific and nonspecific immunological processes (El-Sayed *et al.*, 1995; Rodriguez *et al.*, 2005).

In malnourished children, levels of serum antibody to many antimicrobial antigens (both T cell - dependent and cell-independent) were found to be significantly lowered than normal children. Anemia which is commonly present in PEM further aggravates this immune disturbance (Brussow *et al.*, 1995; Najera *et al.*, 2004).

Opportunistic mycoses in immunocompromised or seriously ill patients are now a familiar problem in many parts of the world. causing an appreciable morbidity and mortality (Hay, 1992).

Malnutrition is considered an important predisposing factor for colonization and infection by these opportunistic pathogens. *Candida albicans* is responsible for the majority of all fungal infections among immunocompromised patients (Eisen and Lynch, 1998). *Candida* spp. is a common commensal of the oral cavity and gastrointestinal tract. Under certain conditions, it may cause either superficial or systemic infections. Systemic infections are seen mainly in immunocompromised patients or following surgery. The organisms usually invade through the gastrointestinal tract or through intravenous lines.

The objective of this study was to assess the prevalence of fungal and bacterial infections in malnourished children and correlating these infections with the type and severity of malnutrition.

MATERIALS AND METHODS

The present study was carried out on 50 infants suffering from PEM. They were 26 males and 24 females. Their ages ranged from 1 to 36 months.

Cases included 25 infants with marasmus and 25 infants with KW, The marasmus group included 13 males and 12 females, their ages ranged from 1 to

20 months while the KW group included also 13 males and 12 females, their ages ranged from 4 to 36 months.

Patients were selected from the outpatient malnutrition clinic of the Center for Social and Preventive Medicine (CSPM) Cairo University.

All patients were subjected to: 1: Full history taking; 2: Thorough clinical examination; 3: Anthropometric measures Weight, length (Ht.) and head circumference; 4: Routine investigations: CBC, urine and stool analysis; 5: Microbiological study urine samples obtained by suprapubic aspiration and both rectal and both rectal and mouth swabs as well as gastric aspirate using nasogastric feeding tube were taken from each patient. All samples were cultured to detect bacterial and fungal pathogens.

Microbiological methods: Specimens were cultured according to the standard procedure of Lennette *et al.* (1985) as follows:

- Gastric aspirates and mouth swabs were cultured on blood, Mac Conkey, chocolate agar supplemented with Vitox (Oxoid) and Sabouraud agar.
- Urine samples were cultured on blood, Mac Conkey and Sabouraud agar.
- Rectal swabs were cultured on blood, Mac Conkey SS and Sabouraud agar.

Identification of the isolated bacterial pathogens: The isolated microorganisms were identified by colony morphology, Gram smear, as well as biochemical and enzymatic reactions including catalase, urease, coagulase, oxidase, bile solubility, CHO fermentation reactions, amino acid decarboxylation on Triple Sugar Iron agar (TSI), lysine iron agar (LIA), motility indole ornithine (MIO) (GIBCO) according to the standard methods (Lennette *et al.*, 1985).

Identification of isolated fungi

Yeast like fungi: The colonies which were small, round, smooth, creamy and have yeast odor were subjected to microscopic examination. If proved to be yeast colonies, it was subjected to germ tube test to identify *C. albicans*. If the yeast cells don't show germ tube, the culture is reported as yeast other than *C. albicans* isolated and identification is done by API 20°C

Mould like fungi were identified by

- Gross colonial morphology
- Microscopic identification by the micro slide culture technique

RESULTS

In the whole cultures of PEM group (n = 200) results revealed that there were (55.5%) positive and 44.5%

Table 1: Culture results of patients with PEM (n = 50)

Cultures	Cultures results		p-value*
	Positive (%)	Negative (%)	
Urine (n = 50)	13 (26)	37 (74)	<0.01
Mouth (n = 50)	30 (60)	20 (40)	NS
Rectal (n = 50)	39 (79)	11 (22)	<0.001
Gastric (n = 50)	29 (58)	21 (42)	NS
Total (n = 200)	111 (55.5)	89 (44.5)	NS

*p-value <0.05 is considered significant, NS = Not Significant

Table 2: Culture results of marasmus patients (n = 25)

Cultures	Cultures results		p-value*
	Positive (%)	Negative (%)	
Urine (n = 50)	5 (2)	20 (80)	< 0.01
Mouth (n = 50)	19 (76)	6 (24)	< 0.05
Rectal (n = 50)	23 (92)	2 (8)	< 0.001
Gastric (n = 50)	18 (72)	7 (28)	< 0.05
Total (n = 200)	65 (65)	35 (35)	< 0.01

p-value <0.05 is considered significant, NS =Not Significant

negative cultures and this was statistically non significant.

Rectal cultures (n = 50): +ve results were obtained in 39 (79%) patients and -ve results in 11(22%) patients. This was statistically significant (p<0.001) (Table 1).

Among the 100 cultures of the marasmus group, +ve results were present in 65% and -ve results in 35%. This was statistically significant (p<0.01).

Mouth cultures (n = 25): +ve results were obtained in 19 (76%) patients and -ve results in 6 (24%) patients and this was statistically significant (p<0.05).

Gastric cultures (n = 25): +ve results were obtained in 18 (72%) patients and -ve results in 7(28%) patients. This was statistically significant (p<0.05) (Table 2).

Among the whole +ve fungal culture results (n = 16), 13 (81.3%) and 3 (18.7%) were present in the marasmus and KW patients, respectively. This was statistically significant (p<0.05).

Candida albicans was present in 11 (84.6%) and 2 (15.4%) of +ve fungal cultures in marasmus and KW groups. This was statistically significant (p<0.05) (Table 3).

Table 3: Type and prevalence of fungal pathogens in relation to the type of malnutrition

Type of malnutrition	Positive fungal cultures			Total (%) (No. = 16)
	<i>A. niger</i> (%) (No. = 2)	<i>C. albicans</i> (%) (No. = 13)	<i>C. tropicalis</i> (%) (No. = 1)	
Marasmus	1 (50)	11 (84.6)	1 (100.0)	13 (81.3)
Kwashiorkor	1 (50)	2 (15.4)	0 (0.0)	3 (18.7)
p-value	NS	< 0.05	NS	< 0.05

Table 4: Type and prevalence of fungal pathogens in relation to all positive cultures of marasmus and kwashiorkor patients

Positive cultures	Fungal pathogens			p-value
	<i>A. niger</i> (%)	<i>C. albicans</i> (%)	<i>C. tropicalis</i> (%)	
Marasmus (No. = 65)	1 (1.5)	11 (16.9)	1 (1.5)	< 0.01
Kwashiorkor (No. = 46)	1 (2.2)	2 (4.3)	0 (0.0)	NS
Total (No. = 111)	2 (1.8)	13 (11.7)	1 (0.9)	< 0.01

Among the whole +ve culture results of PEM (n = 111), *C. A. niger* and *C. tropicalis* were present in 11(16.9%), 2 (1.8%) and 1(0.9%), respectively. This was statistically significant (p<0.01). Concerning the +ve culture results of marasmus (n = 65), *C. albicans*, *A. niger* and *C. tropicalis* were present in 11 (16.9%) and 1 (1.5%), respectively. This was statistically significant (p<0.01) (Table 4).

Among the whole +ve culture results of PEM (n= 111), 43 (38.7%) and 68(61.3%) were present in patients aged <6 months and >6 months respectively. This was statistically significant (p<0.05) (Table 5).

Among the whole +ve cultures of PEM (n = 111), 15 (13.5%), 56 (50.5%) and 40(36%) were present in mild moderate and severe degrees of malnutrition, respectively. This was statistically significant (p<0.01) (Table 6).

Among the +ve cultures of marasmus patients (n = 65), bacterial pathogens were present in 52 (80%) and fungal pathogens were present in 13 (20%) and among the +ve cultures of KW patients (n = 46), bacterial pathogens were present in 43 (93.5%) and fungal pathogens were present in 3 (6.5%). *E. coli* represented the most common offending bacterial pathogen and was present in 56 (50.5%) and *C. albicans* represented the most common offending fungal pathogen and was present in 13 (11.7%) (Table 7).

In marasmus patients, the distribution of *E. coli* (n in different cultures was 23 (71.9%), 7(21.9%), 1(31%) and 1(31%) in rectal, gastric mouth and urine cultures, respectively. The distribution of *C. albicans* (n = 11) in different cultures was 10 (90.9%) and 1(9.1%) in mouth and gastric cultures, respectively.

In KW patients, the distribution of *E. coli* (n = 24) in different cultures was 16 (66.7%), 3 (12.5%), 3 (12.5%) and 2 (8.3%) in rectal, gastric, mouth and urine cultures respectively. *Candida albicans* (n = 2) was present only in mouth cultures (100%) (Table 8).

DISCUSSION

Worldwide, malnutrition is one of the leading causes of morbidity and mortality in childhood (Churran and Barnes, 2000) It is reported to be the most common cause of acquired immunosuppression (Bohler and Wathne, 2000) and this increases susceptibility to infection

Table 5 : Age in relation to positive cultures in patients with PEM

Positive cultures	Age of PEM patients (months)		p-value
	<6 No. (%)	>6 No. (%)	
Total (No. = 111)	43 (38.7)	68 (61.3)	< 0.05

Table 6: Degree of malnutrition in relation to positive cultures in patients with PEM

Positive cultures	Gomez classification (Degree)			p-value
	Mild No. (%)	Moderate No. (%)	Severe n (%)	
Total (No. = 111)	15 (13.5)	56 (50.5)	40 (36)	< 0.01

Table 7: Prevalence of each pathogen in all positive cultures of patients with PEM

Pathogens	Positive cultures		
	PEM (n = 111) No. (%)	Marasmus (n = 65) No. (%)	Kwashiorkor (n = 46) No. (%)
Bacterial			
<i>C. freundii</i>	4 (3.6)	2 (3.1)	2 (4.3)
CONS	3 (2.7)	3 (4.6)	0 (0.0)
<i>E. coli</i>	56 (50.5)	32 (49.2)	24 (52.2)
<i>Enterobacter</i> sp.	2 (1.8)	1 (1.5)	1 (2.2)
<i>H. influenzae</i>	1 (0.9)	1 (1.5)	0 (0.0)
<i>K. pneumoniae</i>	12 (10.8)	5 (7.7)	7 (15.2)
<i>p. aeruginosa</i>	6 (5.4)	2 (3.1)	4 (8.7)
<i>Prov. rettgeri</i>	2 (1.8)	2 (3.1)	0 (0.0)
<i>S. aureus</i>	12 (10.8)	8 (12.3)	4 (8.7)
<i>S. epidermidis</i>	3 (2.7)	2 (3.10)	1 (2.2)
<i>S. faecalis</i>	1 (0.9)	1 (1.5)	0 (0.0)
<i>S. pneumoniae</i>	1 (0.9)	1 (1.5)	0 (0.0)
<i>S. pyogenes</i>	3 (2.7)	1 (1.5)	2 (4.3)
<i>S. saprophyticus</i>	1 (0.9)	0 (0.0)	1 (2.2)
<i>Y. enterocolitica</i>	1 (0.9)	0 (0.0)	1 (2.2)
Fungal			
<i>A. niger</i>	2 (1.8)	1 (1.5)	1 (2.2)
<i>C. albicans</i>	13 (11.7)	11 (16.9)	2 (4.3)
<i>C. tropicalis</i>	1 (0.9)	1 (1.5)	0 (0.0)

Table 8: Prevalence of each pathogen at different positive cultures of kwashiorkor patients

Pathogens	Positive cultures				
	Total No. (%)	Urine No. (%)	Mouth No. (%)	Rectal gastric No. (%)	Gastric No. (%)
Bacterial					
<i>C. freundii</i>	2 (100)	1 (50.0)	0 (0.0)	0 (0)	1 (50)
<i>E. coli</i>	24 (100)	2 (8.3)	3 (12.5)	16 (66.7)	3 (12.5)
<i>Enterobacter</i> sp.	1 (100)	0 (0.0)	0 (0.0)	0 (0)	1 (100)
<i>K. pneumoniae</i>	7 (100)	0 (0.0)	1 (14.3)	0 (0)	6 (85.7)
<i>P. aeruginosa</i>	4 (100)	2 (50.0)	0 (0.0)	0 (0)	2 (50)
<i>S. aureus</i>	4 (100)	1 (25.0)	3 (75.0)	0 (0)	0 (0)
<i>S. epidermidis</i>	1 (100)	1 (100.0)	0 (0.0)	0 (0)	0 (0)
<i>S. pyogenes</i>	2 (100)	0 (0.0)	2 (100.0)	0 (0)	0 (0)
<i>S. saprophyticus</i>	1 (100)	1 (100.0)	0 (0.0)	0 (0)	0 (0)
<i>Y. enterocolitica</i>	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0)
Fungal					
<i>A. niger</i>	1 (100)	0 (0.0)	1 (100.0)	0 (0)	0 (0)
<i>C. albicans</i>	2 (100)	0 (0.0)	2 (100.0)	0 (0)	0 (0)

(Christie *et al.*, 1992). In severely malnourished children, respiratory, urinary and other infections are common but are not easily diagnosed because the classical signs of infection (fever, pain, inflammation etc.) may be masked because the immune system is inhibited and the child doesn't have the normal defense mechanisms. Severely malnourished children can develop septicemia without fever (WHO, 2000).

In this clinical study over a 2 years period, we attempted to elucidate the prevalence of fungal and bacterial infections in infants and children with PEM. Among 200 cultures done, 111 were positive (55.5%) for various pathogens. Fungi were detected in 14.4% of these positive cultures, The yield of positive cultures was higher in rectal (35.1%) and mouth (27.1%) than in gastric (26.1%) and urine (11.7%).

The severity of malnutrition determines the incidence of infections and is considered a risk factor for mortality (Teka *et al.*, 1996). Present results revealed that most positive cultures (86.5%) were present in moderate- severe degrees of malnutrition.

Gram negative aerobes were isolated more frequently from malnourished than from well-nourished children (Friedland, 1992). In this study *E. coli* was the most prevalent organism (50.5, 49.2 and 52.2%) isolated from positive cultures of PEM, marasmus and KW patients respectively. This can be explained by Kaijser *et al.* (1977) who found that strains of *E. coli* elaborate envelop or capsular acidic polysaccharide antigen called K-antigen. This specific K-antigen is associated with *E. coli* strains implicated in infection of many different tissues including those of urinary tract.

Candida species and in particular *C. albicans* represent a serious threat to immunosuppressed patients and it appears to be related to suppression of cell-mediated immune response against this pathogen (Arnold *et al.*, 1995; Najera *et al.*, 2004). This is in agreement with the present results where *C. albicans*

was the most prevalent fungal pathogen (11.7, 16.9 and 4.3%) isolated from positive cultures of PEM, marasmus and KW patients, respectively.

Caksen *et al.* (2000) found that in infants with malnutrition, Gram-negative microorganisms are the predominant pathogens in urinary tract infections.

The high incidence of urinary tract infection in PEM could be attributed to a defect in the local antibody response including locally synthesized IgG and secretory IgA that are normally present in urine and whose competences inhibit bacterial adhesion to the epithelial surface (Chandra, 1992). In the present study the incidence of urinary tract infection was 26, 20 and 32% among PEM, marasmus and KW patients, respectively. Culture results go hand-in-hand with the results of Daoud (1994) who found UTI in 28% of patients with PEM. In the present study, there was a female preponderance among cases (69.2 versus 30.8%). The occurrence of UTI was reported to be higher in females and among uncircumcised males (Elder, 2000). *E. coli* is one of the predominant Gram-negative organisms in UTI among children with PEM (Isaak *et al.*, 1992). In our study, *E. coli*, *P. aeruginosa* and *S. epidermidis* constituted 69.2, 60 and 62.5% of positive urine cultures of PEM, marasmus and KW patients respectively. *E. coli* was present in 23.1% of positive urine cultures of PEM. This result goes hand-in-hand with Berkowitz (1983) who found *E. coli* in 31% of positive urine cultures of PEM.

Malnutrition elicits adverse alterations in the oral microbial ecology as well as in the volume, antimicrobial and physico-chemical properties of saliva (Enwonwu, 1995). It predisposes also to carriage of oral yeast and subsequent candidiasis (Matee *et al.*, 1995). Moreover, it enhances the presence of yeast species other than *C. albicans* (Jabra *et al.*, 2001).

In the present study, positive mouth cultures were present in 60, 76 and 44% of PEM, marasmus and KW patients, respectively. In PEM patients, bacterial pathogens represented 60.5% and fungal pathogens represented 39.5% of positive mouth cultures. Mixed mouth infections were detected in 23.3, 31.6 and 9.1% of the same groups of patients, respectively. These mixed cultures may point to the effect of malnutrition on the immune system, paving the way for more than one pathogen to cause infection at the same site (Redmond *et al.*, 1991).

According to the study done by Shaaban *et al.* (2001) who noted that gastric emptying is delayed in PEM by ultrasonography, we used gastric aspirate as

representative of organisms coming from the respiratory tract. Acute respiratory infections are among the leading causes of childhood mortality (Williams *et al.*, 2002). In developing countries, malnutrition, lack of breast feeding, low socioeconomic status and sharing of sleeping space are important risk factors for developing respiratory tract infection (Victora *et al.*, 1999; Nascimento-Crvalho, 2002).

In the present study, positive gastric cultures were present in 58, 72 and 44% of PEM, marasmus and KW patients respectively. Bacterial pathogens represented 97% and fungal pathogens represented 3% of positive gastric cultures. *E. coli* and *Klebsiella pneumoniae* were the most prevalent bacterial pathogens detected and *C. albicans* was the only fungal pathogen detected in positive gastric cultures of malnourished children.

Malnourished children have significantly higher incidence of diarrhea disease even after controlling the possible confounding history of illness (Lindijorn *et al.*, 1993). Also they may have the tendency to persistent diarrhea than healthy ones due to impaired cellular immunity (Taniguchi *et al.*, 1999). This persistent diarrhea in turn aggravates the nutritional status and may cause zinc deficiency which may worsen this diarrhea (Bohler and Wathne, 2000). Significant weight loss is reported during symptomatic intestinal infection with cryptosporidium and enteroaggregative *E. coli* (Bahn *et al.*, 2000). *Yersinia enterocolitica* can cause illness ranging from self-limited enteritis to life-threatening systemic infection (Abd El-Hag *et al.*, 2000). In the present study, positive rectal cultures were present showing that *E. coli* was the most prevalent bacterial pathogen detected, *Y. enterocolitica* was detected in only 2.5% of positive rectal cultures of PEM patients.

The predominance of *E. coli* in rectal cultures may be explained by (Kurioka *et al.*, 1998) who found in animal studies that PEM severely affected the development of intestinal physical barrier (epithelial) and this is considered one of the predisposing factors for infection with certain strains of *E. coli* (shiga toxin producing *E. coli*). This may explain the high incidence of infection with *E. coli* in PEM children.

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