

The Prevalence of *Candida albicans* - Associated Diarrhoea in Buea, South West Cameroon

Theresa K. Nkuo-Akenji*, Roland N. Ndip and Fritz A. Ntoko

Department of Life Sciences, Faculty of Science, University of Buea, P.O. Box 63 Buea, S.W. Province, Cameroon.

* To whom correspondence should be addressed.

SUMMARY

A total of 362 stool specimens were collected from 184 and 178 patients presenting at the Buea district Hospital with and without diarrhoea, respectively. The samples were screened and cultured for *Candida albicans* using standard microbiological procedures. Of the 184 diarrhoeic stool cultures, 35.9% showed *C. albicans* overgrowth as indicated by count $\geq 10^4$ CFL/mL. Of the 178 non diarrhoeic stool cultures, *C. albicans* was identified in 23.6% of samples and counts were all $< 10^4$ CFU/mL. An association was observed for *C. albicans* overgrowth and diarrhoea ($p < 0.001$). The majority of isolates (87.8%) from the 66 samples showing *candida* overgrowth were susceptible to Amphotericin B in anti-fungal drug sensitivity assays. Results of the study highly suggest that *C. Albicans* is an important cause of diarrhoea in the study area. We recommend that this fungus should be routinely checked in individuals presenting with diarrhoea particularly children and patients on prolonged or frequent antibiotic therapy.

[Afr. J. Health Sci. 2002; 9:153-157]

Introduction

Diarrhoea is responsible for a substantial degree of morbidity and mortality among children and adults in both developing and developed countries [1]. Diarrhoea has been reported to be the second leading cause of death in children world wide and accounts for 2.9 million child deaths [2]. Various microbial agents have been incriminated in diarrhoea among which are enteric bacteria, parasites and viruses [3, 4, 5]. *Candida*, *Trichosporon* and *Geotrichum* are fungi that have been reported to cause diarrhoea [6, 7]. *Candida albicans* is an opportunistic pathogen of humans. This fungus can take advantage of a depressed immune system, a state of hormonal imbalance, increased sugar level, stress and consumption of broad-spectrum antibiotics to become

pathogenic. Under these conditions *C. albicans* could grow and cause diarrhoea.

Research in the area of fungal diarrhoea is lacking in Cameroon. This study therefore reports on the prevalence of *C. albicans* - associated diarrhoea in the Buea district and has provided additional important information for better control and management of diarrhoea.

Materials and Methods

Study population

A total of 362 individuals comprising 175 females and 187 males aged 2 months to 72 years old attending health facilities in the Buea district, were recruited into the study. Of these 184 had diarrhoea and 178

had no diarrhoea. The medical history of patients was obtained from medical records and from the administration of a questionnaire. These provided information on factors, which may influence the growth of *Candida* such as the frequency of antibiotic consumption, the use of contraceptive, and the presence of clinical conditions such as diabetes mellitus, fever and abdominal pain. A questionnaire was also administered to 18 medical doctors to determine how many of them were aware of *C. albicans*-associated diarrhoea and how many prescribed antifungal agents against diarrhoea.

Sample Collection

Stool samples were collected from individuals with and without diarrhoea into sterile containers, which were appropriately labeled. All samples were transported to the laboratory at the University of Buea within one hour of collection and refrigerated until the time of analysis (within 12 hrs of collection).

Isolation and identification of C. albicans

Approximately 0.1 ml (or 0.1g of formed stool) of stool sample was diluted in 0.9 ml of sterile distilled water. Serial dilutions were made up to 10^8 . A volume of 0.1 ml from each dilution was spread on Sabouraud's dextrose agar to which chloramphenicol had been added (0.05 mg/1mL) to make it more selective. The plates were incubated at 37°C for 24-48 hours. Suspected colonies were streaked onto fresh agar to obtain pure colonies. *C. albicans* colony count was done on the primary culture after identification of representative colonies. Counts of $\geq 10^4$ cfu/ml were considered as overgrowth [7]. Further identification of isolates was based on germ tube formation, pattern of growth on corn meal agar, carbohydrate fermentation and carbohydrate assimilation.

Susceptibility testing

The susceptibility of *C. albicans* isolates to antifungal drugs was performed using the disk diffusion method [8]. The drugs tested were nystatin (25µg), amphotericin B (25µg), griseofulvin (25µg), and clotrimazole (25µg).

Statistical analysis

The statistical software EPI INFO6 was used for statistical analysis. Chi-square and two-tailed Fischer exact test were used for categorical analysis where appropriate (p values < 0.05 were considered significant).

Results

Prevalence of Diarrhoea

A total of 362 individuals took part in the study of which 187 and 175 were males and females respectively. Their ages ranged from 2 months to 72 years. The prevalence of diarrhoea was 50.83% (98 and 86 males and females respectively).

Frequency of Isolation of Candida albicans

Of 362 stool samples analyzed, 122 (33.7%) isolates of *C. albicans* were identified. Among the 184 samples from patients with diarrhoea, 66 (35.87%) showed an overgrowth of *C. albicans* ($\geq 10^4$ cfu/ml). Of the 118 considered to have non-*Candida* associated diarrhoea, samples from 14 of these showed growth $< 10^4$ cfu/ml while 104 samples were negative for *Candida* (Table 1). Of the 178 samples from patients presenting with no diarrhoea, 42 (23.6%) had *C. albicans* but the counts were all $< 10^4$ cfu/ml while the rest were negative. There was a significant relationship between *C. albicans* overgrowth and diarrhoea ($p < 0.01$).

Relationship of age and Candida-Associated Diarrhoea (CAD)

The prevalence of diarrhoea and of CAD in the study population by age group is shown in Table 2. There was a highly significant relationship ($p < 0.01$) between the prevalence of diarrhoea and age group.

Similarly, a significant relationship ($p < 0.05$) was found between CAD and age. The highest prevalence of diarrhoea and of CAD was observed in children 10 years of age and younger

Association of CAD and some underlying conditions

A significant relationship ($p < 0.001$) was found to exist between CAD and prolonged use of antibiotics and also pruritis ani (Table 3).

Antifungal Susceptibility testing

The susceptibility pattern of 66 *C. albicans* Isolates obtained from patients with CAD showed that 87.79% of isolates were susceptible to amphotericin B. Analysis of the questionnaire administered to 18 medical doctors revealed that in the treatment of diarrhoea, yeast cultures were hardly ever requested. The majority of doctors (66.67%) prescribed antifungal drugs only when patients presented with oral thrush or were suspected to be immunosuppressed.

Table 1. Relationship between *C. albicans* overgrowth and diarrhoea

CASES	C.ALBICANS COUNT (cfu/ml)		TOTAL
	<10 ⁴	>10 ⁴	
Non-diarrhoea	178	0	178
Diarrhoea	118	66	184
Total	296	66	362

$X^2 = 77.87$; $df = 1$; $p < 0.001$

Table 2: Prevalence of diarrhoea and of CAD by age group

Age Group (Years)	No. of Individuals	Prevalence of Diarrhoea (%)*	Prevalence of CAD (%) ⁺
<10	116	69.83	25.86
11.20	83	34.94	12.05
21.30	72	37.50	12.50
>30	91	51.65	18.68

* $X^2 = 30.28$; $df = 3$; $p < 0.01$ + $X^2 = 8.25$; $df = 3$; $p < 0.05$

Table 3: Association between CAD and some underlying conditions

Feature/Factor	Patients with >10 ⁴ CFU		Patients with <10 ⁴ CFU/mL		p-value
	+	-	+	-	
Antibiotic intake	39	27	65	231	<0.001
Oral Contraceptives	3	63	02	294	<0.05
Diabetes	2	64	1	295	<0.05
Abdominal pain	27	39	76	220	<0.01
Pregnancy	7	59	12	284	0.05889
Fever	32	34	109	187	0.0821
Vomiting	1	65	10	286	0.6967
Anorexia	8	58	13	283	0.0351
Pruritis ani	16	50	2	294	<0.001

+ Presence of feature - Absence of feature

Discussion

The most common symptom indicating an infection of the lower intestinal tract is diarrhoea. The high frequency of diarrhoea cases in developing countries has been associated mainly with bacteria, viruses and parasites. Extensive studies have not been done in the area of fungal diarrhoea. The data obtained from our study revealed that of 184 patients with diarrhoea, 66 (35.87%) had *Candida albicans* associated diarrhoea (CAD) as indicated by an overgrowth of $\geq 10^4$ cfu/ml. Danna and collaborators [7] reported *C. albicans* overgrowth in 30% of elderly patients who were on antibiotics and had diarrhoea. In another study involving 203 children (0-10 years old) with diarrhoea, a higher prevalence of 48.28% was reported [9] although there was no dichotomy of colony counts made. The prevalence of *C. albicans* in non-diarrhoeal individuals in the Buea district was 25.59% but the stool specimens from these individuals showed counts < 10 cfu/ml. This is expected, as *C. albicans* is also a member of the normal intestinal flora.

Data obtained from this study revealed a significant relationship ($p < 0.001$) between *C. albicans* overgrowth and diarrhoea. There was no significant association ($p > 0.05$) between the occurrence of CAD and sex although its prevalence was higher in males (55.55%) than in females (45.45%).

This observation is consistent with that of Enweani and collaborators [9]. The occurrence of CAD was significantly higher in children less than 10 years old. This group also had the highest prevalence of diarrhoea.

Several factors thought to influence the growth of *Candida albicans* were investigated. The practice of auto-medication in the community has reached alarming proportions and is cause for concern particularly as some of the antibiotics readily available from street vendors are broad spectrum antibiotics and individuals usually administer these in wrong doses. A significant association ($p < 0.001$) was found to exist between antibiotic consumption and CAD.

Antibiotics suppress the normal flora and allow *C. albicans* hyperproliferation. The use of oral contraceptives was also associated with CAD ($p < 0.05$) although the number of participants on contraceptives was rather small. Oral contraceptives contain oestrogen and progesterone, which may cause hormonal imbalance and favour overgrowth of *C. albicans*. Increase in glucose levels has been reported to precipitate candidiasis [10]. Although only 2 diabetic cases were identified in the study population, they all had CAD. Abdominal pain as a clinical feature was associated with CAD. Pruritis ani, which is characterised by severe itching and irritation of the anus due to colonization by *Candida* and its metabolic products, was observed in 16 patients with CAD.

There have been reports on resistant of fungi to commonly used anti-fungal drugs [11]. Susceptibility testing of 66 *C. albicans* isolates to several anti-fungal agents showed resistance levels of 57.58% and 31.81% to griseofulvin and clotrimazole respectively. Amphotericin B had the lowest resistance level. It was observed that some medical doctors did not request for laboratory investigations of *C. albicans* as this organism is not considered to be a potential cause of diarrhoea.

Conclusion

Results of this study revealed a strong association between diarrhoea and *C. albicans* overgrowth, indicating an important aetiologic role of the organism in diarrhoea. An association between CAD and some underlying conditions particularly prolonged antibiotic intake was also established. Amphotericin B was identified as the drug of choice against *C. albicans*. Results of the study indicate that there is a need for better laboratory diagnosis and management of diarrhoea.

Acknowledgements

Special thanks go to the medical staff of the Buea General Hospital for their assistance.

We are grateful to the University of Buea for funding the work.

References

1. Alabi SA and Odugbemi T. Occurrence of *Aeromonas* species and *Pleiomonas shingelloides* in patients with and without diarrhoea in Lagos Nigeria. *Journal of medical Microbiology*. 1990; 32: 45-48
2. Grant JP. The state of the world's children. London: Oxford University Press 1994: pp. 4-9
3. Henry FJ. Combating childhood diarrhoea through international collaborative research. *Journal of Diarrhoeal Disease Research*. 1991; 9: 165-167.
4. Babaniyi OA. Oral dehydration of children with diarrhoea in Nigeria: a 12-year review of impact on morbidity and mortality from diarrhoea diseases and diarrhoeal treatment practices. *Journal of Tropical Pediatrics*. 1991; 37: 57-62.
5. Bignan FU, Unicomb L, Rahim Z, Banu NN, Podder G and Clemens J. Rotavirus associated diarrhoea in rural Bangladesh: two-year study of incidence and serotype distribution. *Journal of Clinical Microbiology*. 1991;7:1359-1363.
6. Talwar P, Chakrabarti A, Chawla A, Mehta S, Walia BNS, Kumar L and Chugh KS. Fungal diarrhoea: association of different fungi and seasonal variation in their incidence. *Mycopathology*. 1990; 110: 101-105.
7. Danna PL, Urban C, Bellin E and Rahall JJ. Role of *Candida* in pathogenesis of antibiotic-associated diarrhoea in elderly patients. *Lancet*. 1991; 337: 511-14
8. Bauer AW, Kirby WW, Sherris JC and Tenckhoff M. Antibiotic susceptibility testing by standardized single disc method. *American Journal of Clinical Pathology*. 1966; 36: 493-96
9. Enweani B, Obi CL and Jokpeyibo M. Prevalence of *Candida* species in Nigerian children with diarrhoea. *Journal of Diarrhoeal Disease Research*. 1994; 12: 133-35.
10. Chopia HL. Candidiasis. Textbook of Medical Microbiology. 1st edition. Delhi: Seema Publications, 1985: 1719-1725.
11. Odds FC. Resistance of yeast to azole derivative antifungals. *Journal of Antimicrobial Chemotherapy*. 1993; 31: 463-471.

Manuscript received on: 20 April 2002; Approved for publication on: 15 June 2002