INTESTINAL ORGANISMS AND ENTEROPATHY IN HIV RELATED DIARRHOEA AND THEIR RESPONSE TO ALBENDAZOLE THERAPY

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DECLARATION

I hereby declare that the work presented in this study for the Master of Medicine degree is original and has not
been presented either wholly or in part for any other degree and is not being currently submitted for any other
degree.

Signed. _____
Student

Signed. _____
Supervisor

Date: 2/4/98.

APPROVAL

THIS DISSERTAION OF ISAAC STEPHEN ZULU

HAS BEEN APPROVED AS A PARTIAL

FULFILLMENT OF THE REQUIRMENTS FOR THE

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DEDICATION

This dissertation is dedicated to My Late Father

Stephen Mwale, My Mother and

My Wife Elizabeth

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SUMMARY

Treatment of HIV related diarrhoea has been a problem due to lack of effective chemotherapeutic agents against the different organisms responsible for the problem. Following the promising results of a double blind randomised placebo controlled study at the University Teaching Hospital , Lusaka , Zambia which showed that 50% of HIV positive patients with persistent diarrhoea (diarrhoea of one month or more in duration) and Karnofsky's score between 50 and 80 responded to 2 weeks of Albendazole therapy, a study was undertaken to establish the organisms that were eradicated by the chemotherapeutic agent. This study directly follows the previous Albendazole study which did not look at the specific organisms eradicated by the therapy. Bacterial profile of the group of patients studied was looked at and response of HIV - related enteropathy was also studied. 83 HIV positive patients with persistent diarrhoea were studied. After 2 weeks of Albendazole 800 mg twice a day, 33 (40%) patients had complete response , 29 (35 %) had partial response and 21 (25%) did not have any response . Albendazole eradicated *Microsporidium* in 73 % of patients , *Isospora belli* in 41 % of patients and *Crypotosporidium* in 100 % of a small number of patients . *Isospora belli* was eradicated in 5 (28%) out of 18 patients by Co trimoxazole. 10 (55 %) of this group of patients experienced complete clinical response and 5 (28 %) had partial response. 3 (17 %) did not experience any response. Patients with higher CD4s showed better clinical response. Regeneration of the mucosal villi did not occur after parasites eradication.

INTRODUCTION

HIV INFECTION AND GASTROINTESTINAL DISEASE IN ZAMBIA

The HIV epidemic is a major health problem in the world today especially in Sub- Saharan Africa. The new epidemic has brought with it new disease patterns which include infectious conditions caused by organisms which previously were not major health concerns. Persistent diarrhoea (diarrhoea of one or more months' duration) is one of the most common manifestation of HIV. It is largely caused by organisms like *Cyptosporidium*, *Microsporidia*, *Isospora belli* which until the emergence of HIV were not recognised to be organisms of great medical significance. Diarrhoea is a major problem in adults with AIDS in Sub - Saharan Africa . In Lusaka , Zambia , 1 % of the adult population has persistent diarrhoea at any given time (Kelly et al 1995). Studies done in Zaire , Uganda and Tanzania show that the occurrence of diarrhoea in AIDS varied from 40 - 75 % (Colebunders R L et al. 1991) . Associated with diarrhoea is an entity called enteropathy . Enteropathy refers to structural and functional changes in gut mucosa in response to local or systemic factors. Reduction in villous height and increase in crypt depth occur. There is sometimes hyperplasia of cells of inflammation.

LITERATURE REVIEW

Studies done in Zambia and elsewhere show that *Cryptosporidium*, *Microsporidium* and *Isospora belli* are emerging as the three commonest causes of HIV - related persistent diarrhoea.

Two previous studies by Conlon et al 1990 and Kelly et al 1996 have shown that parasitic infections are a major contributor to the HIV- related persistent diarrhoea. Conlon's study demonstrated that *Cryptosporidium, Isospora belli* and *Strongyloides stercolaris* were the common organisms found in 32 %, 16 % and 6 % of HIV related diarrhoea cases respectively. This study was the first in the University Teaching Hospital to try to establish the organisms involved in HIV - related diarrhoea . Kelly et al , demonstrated that *Microsporidium , Isospora belli, Cryptosporidium* and *Blastocystis hominis* were the common organisms in HIV-related diarrhoea. Each of these was isolated in 35 %, 28 %, 25 % and 11 % of cases respectively in 75 HIV - seropositive individuals studied at the University Teaching Hospital (Kelly et al 1996) . Studies done elsewhere have also shown the increased prevalence of the protozoal infections identified in Lusaka. In preliminary studies done in Uganda , *Cryptosporidium* and *Isospora belli* were common in stools of patients with slim disease. In the United States and Europe, *Cryptosporidium* and *Isospora belli* are found in stools of up to 15 % of homosexual men with HIV - related diarrhoea

(Conlon et al 1990). The various studies in different parts of the world demonstrates the rising importance of *Cryptosporidium* , *Isospora belli* and *Microsporidium* in HIV infection as the leading organisms in causing diarrhoea . In the pre HIV era *Cryptosporidium* and *Microsporidium* were responsible rarely for an acute self limiting diarrhoea.

CRYPTOSPORIDIUM

Cryptosporidium is a protozoan first identified in 1907. It is an intracellular parasite abiding at the microvillous border of the intestinal epithelium. The organism was initially thought to be non - pathogenic until 1955 when a fatal diarrhoea syndrome in turkeys was described . The first diarrhoea reports in man were in 1976 (Kelly P , 1997). Its identification in AIDS patients in the 1980s established its role as an opportunistic infection.

The organism has notable epidemic potential. There have been recent waterborne outbreaks of diarrhoea caused by the organism such as occurred in Milwaukee, Wisconsin in April 1993 in which an estimated 370 000 individuals were affected (Hayes et al 1989, Terry 1993). Acute self limited diarrhoea is the most common expression of infection in humans . With the advent of HIV, persistent diarrhoea due to the parasite is becoming increasingly a major health problem.

MICROSPORIDIA

Microsporidia are obligate intracellular protozoal organisms. They have a wide host range including most invertebrates and all classes of vertebrates. There are almost 1000 species of the organisms and five have been identified as causing a diverse range of disease in humans. *Enterocytozoon bieneusi* and *Septata intestinalis* are important species causing diarrhoea in humans (Weber et al , 1994). *E. Bieneusi* is the commonest of the two with reported infection rates of 20 - 25 % of patients with HIV- related diarrhoea (Curry A, 1995). The two species can also occur simultaneously (Blanshard C et al , 1992) . This organism is now being isolated more commonly in HIV - related persistent diarrhoea around the world. Earlier studies in Africa showed a low incidence of the organism. As Curry A noted, by 1995 there seemed to be a lower incidence of the organism in Africa than in the developed countries (Curry A ,1995) . Among the early reports of microsporidiosis in Africa is that by Lucas et al (1993) who reported 5 cases of *Enterocytozoon bieneusi* in 77 AIDS patients from Uganda and Zambia with chronic diarrhoea and wasting. In a study of 102 AIDS patients in Bujumbura with chronic diarrhoea *E. Bieneusi* was found in only two patients (Canning EU et al 1993). Conlon did not report any *Microsporidia* in his study at the University Teaching Hospital , Lusaka , Zambia (Conlon 1990) . Subsequent studies done at the same institution have shown a higher incidence of the organism . Kelly et al found the organism in 35 % of the 75 HIV positive patients with persistent diarrhoea (Kelly et a

1996). Richmond V found the organism in 16 % of the 140 HIV positive patients with persistent diarrhoea (personal communication , 1997) . It is not known how intestinal *Microsporidium* is acquired but it is likely that it is by the faeco - oral route and possibly by contamination of water supplies.

ISOSPORA BELLI

Isospora belli is an intracellular coccidian parasite. It is commonly found in tropical and subtropical countries. It is found in the intestines and is responsible for human coccidial infections. The organism has been recognised for more than a century. Human infection however is very rare in immunocompetent individuals (Trier et al 1974) . Infection with this organism is becoming increasingly common in AIDS patients as an opportunistic infection responsible for chronic watery diarrhoea and weight loss (De Hovitz et al , 1986). The organism is spread by faecal- oral route (Farthing 1993), and outbreaks can occur in family affairs or groups exposed to a common source of mature oocysts (Medical letter on Drugs and Therapeutics Handbook of Antimicrobial Therapy Revised Edition 1988).

THERAPY

The major challenge that has arisen with the increasing incidence of diarrhoea caused by these organisms is therapy. Treatment options for the parasites are limited. Effective antiparasitic therapy for *Cyrptosporidium* has been elusive . Currently there is no established effective chemotherapeutic agent. Spiramycin showed early promise but has not stood the test of time . Paromomycin and azithromycin in some model systems studies have been suggested to have some therapeutic potential . AZT may have some benefit as shown by AIDS patients who have shown improvement while on the drug (Farthing 1993).Chemotherapy for *Isospora belli* has been seen not to be predictable in eradication of the parasite. Recurrence of upto 50 % after eradication have been experienced (Farthing 1993). Various agents have been tested and have shown antiparasitic potential. Pape et al successfully treated 32 AIDS patients with one double strength Septrin tablet four times daily . Pyrimethamine has also shown promise in the treatment of the organism (Pape et al , 1989). Diclazuril is another chemotherapeutic agent that has been tested and has been shown to have some activity against the organisms (Kayembe et al , 1989).

Search for an effective agent against *Microsporidium* is also on going. Eeftinck Schattenerk et al (1991) has reported improvement in chronic diarrhoea caused by *Microsporidium* after therapy with Metronidazole. Response against

metronidazole unfortunately is not predictable and this has called for more work to find an agent that is effective (Farthing , 1993). Albendazole has been reported to have efficacy on *Microsporidium*. Blanshard et al (1992) used Albendazole 400 mg for a month to treat 6 consecutive patients with persistent diarrhoea. Diarrhoea completely stopped in these patients. However relapse occurred after 1-3 months after cessation of therapy and this is a major problem in treatment of these organisms. Kelly et al , 1996 showed in a double blind randomised placebo controlled study at the University Teaching Hospital , Lusaka , Zambia that 50% of HIV positive patients with persistent diarrhoea and Karnofsky's score between 50 and 80 responded to 2 weeks of Albendazole therapy . The study however did not look at the parasitological activity of the drug on the three common organisms associated with HIV - persistent diarrhoea.

In the quest for an effective agent against the protozoal organisms a study was designed to observe the effect of Albendazole on the parasites associated with HIV - related diarrhoea. Albendazole was chosen because of its broad spectrum of activity and the fact it has shown some promise in treating *Microsporidia*

ENTEROPATHY

Enteropathy has long been observed in a number of conditions involving the gastrointestinal tract. However its real clinical significance and the mechanism of development is still not very clearly understood. There are various disease conditions in which enteropathy occurs. These have been divided into five major groups ; prolamine hypersensitivities, infective/parasitic, tropical sprue , graft - versus host disease and transient food sensitivities.

Coeliac disease

In prolamine hypersensitivities there is a heightened immunological response in genetically predisposed individuals. Prolamine hypersensitivity occurs in coeliac disease. In coeliac disease, there is an abnormal immunological response to gluten protein which is found in wheat , barley , rye ,oats in genetically predisposed (DQW 2) individuals leading to villous atrophy.

Infectious enteropathy

Infections are a major group of conditions associated with enteropathy. Repeated enteral infections and continuous exposure of the gut to micro-organisms has been shown to be associated with changes in gut structure and function ,which has been described as chronic environmental enteropathy. This persists as long as the individual lives in the unfavourable environment and takes months to improve after moving to a clean environment (Bunser O et al. 1987 , Lindenbaum J et al. 1971). It is likely , but not proved, that chronic environmental enteropathy

reflects response to unaccustomed quantities of environmental bacteria and their products.

A number of parasitic infections have been shown to cause flattening of gut villi . In *Giardia* infestation , there are structural and functional abnormalities mainly in the proximal small intestines. The majority of patients have mild or moderate partial villous atrophy with less than 10% having subtotal villous atrophy. There is usually an associated mild or moderate increase in crypt depth. Even in the absence of changes in villous and crypt architecture, ultrastructural changes such as shortening and disruption of microvilli have been reported (Farthing , 1996). In presumed HIV negative individuals the mucosal abnormalities return to normal following treatment .

Studies done in Puerto Rico (Sheehy T.W. et al. 1962) and India (Burman et al. 1970) showed that in hookworm infestation structural abnormalities of the villi occur which revert to normal after deworming. (Manson's Tropical Diseases, 13th ed, 1996).

Tropical enteropathy

Structural and functional abnormalities in the intestinal mucosa have as well been recognised in tropical sprue and Tropical enteropathy. Tropical enteropathy a condition recognised through out the developing world including Zambia is characterised by asymptomatic jejunal abnormalities and xylose malabsorption in apparently healthy people (Thomas G et al 1976 , Vetch A.M. et al . 1996).

Nutrition

Enteropathy has also been observed in Malnutrition. It was recognised many years ago that the small intestinal walls of malnourished children are very thin probably due to thinning of muscular layers of other wall tissues

(Sullivan P.B. et al. 1992 , Brunser O et al.). However , the mucosal changes could be due to associated infections. No studies in pure malnutrition like anorexia nervosa have been done.

Graft - versus - host disease

Another condition in which enteropathy occurs is graft - versus - host Disease. It occurs in both acute and chronic state of the disease. In the acute state of the disease, there is individual crypt cell destruction . Crypt abscesses may occur finally leading to a totally denuded mucosa. Lymphocytic inflammatory infiltrate is often not marked . In chronic graft - versus - host disease , there are three distinct abnormalities detected ; epithelial cell damage , a mononuclear cell inflammatory infiltrate predominantly of CD8 cells and subepithelial fibrosis

(Atkinson K. 1993.).

HIV - related enteropathy

In HIV infection and in patients with HIV related persistent diarrhoea various studies have demonstrated the presence of enteropathy . Conlon et al and Kelly et al in separate studies have demonstrated the presence of villous blunting and inflammation on duodenal histology in HIV infected Zambian patients with chronic diarrhoea (Conlon C. et al. 1990, Kelly et al. 1996). There are two theories to account for enteropathy in HIV: 1. opportunistic infections 2. HIV itself. However , there is only indirect evidence for the latter.

Conlon and Kelly's studies have associated the enteropathy with opportunistic infections like *Isospora belli*, *Cryptosporidium*, *Microsporidium* which are the major causes of diarrhoea in this group of patients studied in Lusaka, Zambia. Studies done in various parts of the world have as well demonstrated structural and functional changes in duodenal and jejunal mucosa in HIV infection. Cummins A.G. et al. demonstrated enteropathy in eight HIV infected Australian patients with AIDS/AIDS related complex . Villous atrophy with impaired crypt hyperplasia of duodenal mucosa was seen in these patients (Cummins A.G. et al . 1990). A study by Ullrich R in two major hospitals in Berlin showed impairment of function , low grade small bowel atrophy and maturational defects in enterocytes in HIV infected patients. (Ullrich R et al.).

Enteropathy has also been observed in the intestinal mucosa of HIV infected individuals with diarrhoea in whom there is absence of the diarrhoea causing parasites . It has been suggested that probably the virus itself may cause the enteropathy or other unrecognised parasites, or some parasites which have escaped detection in these individuals (Farthing et al ,1996).

Molecular and cellular pathogenesis of enteropathy

The mechanism of development of enteropathy in the various groups is not clearly understood. There are some processes which have been observed and may contribute to the development of the entity. In coeliac disease for example, there is ample evidence for the involvement of the immune system in the amplification and perpetuation of morphological

and functional abnormalities of intestinal mucosa. Gliadin antibodies could play some role in the pathogenesis of the disease although their actual role remains unclear. Gluten has been shown to induce IgG - mediated subepithelial complement fixation. It might via immunoglobulin - mediated subepithelial complement activation damage the surface epithelium . Gliadin antibody could also cause intestinal injury through a cell - mediated cytotoxic reaction in which the antibody recognises gliadin peptides bound to mucosal structures directing an antibody-dependent cell mediated cytotoxic reaction (Maki M, 1995 .). Kontakou M et al and Halstensen TS have all found that immune response to gliadin do seem to occur in lamina propria of coeliac disease patients supporting cell -mediated immunity in the pathogenesis of the disease (Kontakou et al 1995 , Halstensen T S et al 1992) .

In HIV infection , the mechanism of the enteropathy could be due to either the direct effect of the parasite or the immunological response to the infection . In *cryptosporidium* infection , a variety of architectural abnormalities in the small intestines have been observed which range from partial to subtotal villous atrophy (Argenzio et al ,1990 ; Phillips et al 1). Microvillous membrane disruption is also seen especially at the point where the parasite adheres to the mucosa. In infections with *Isospora belli* morphological changes are also observed in the small intestinal mucosa which range from mild to subtotal villous atrophy. The parasite themselves could therefore be the direct cause of the intestinal abnormalities . In addition to the probable effect of the parasites , the infections are associated with mucosal inflammatory infiltrates of lymphocytes, plasma cells and eosinophils which may contribute to mucosal injury and dysfunction (Farthing M J G. 1993.). However , this immune cell infiltration is not associated with T cell activation and

this therefore is unlikely to be responsible for enteropathy (Veitch et al 1995 AGA) .

Significance of enteropathy

Enteropathy is an important entity in the diseases where it occurs as the causation of some of the manifestations of the diseases. In coeliac disease a number of manifestations are a result of the impairment of structure and function of the mucosa. In adults, impairment of absorption will cause biochemical abnormalities in the blood. Low levels of haemoglobin, calcium, potassium , magnesium and iron are significantly more common in patients with the classical symptoms of malabsorption. Microcytic anaemia resulting from impaired iron absorption in the duodenum is quite common and may be the only manifestation of coeliac disease. (Corazza et al , 1995).

In Giardiasis, diarrhoea and malabsorption which occur have been attributed partly to the abberation of epithelial structure and function in the intestinal mucosa. The possible mechanisms of pathogenesis of diarrhoea and malabsorption suggested are ultrastructural damage of microvilli, reduction in villous height and immature enterocytes. Reduction in disaccharidase activity and mucosal inflammation are other possible mechanisms. In tropical enteropathy a similar situation has been observed where reduction in jejunal absorptive capacity occurs (Cook CD, 1980) leading to disturbances like low vitamin B 12 levels.

From what has been observed in the various types of enteropathies described , it is highly likely that the enteropathy in HIV contributes a

great deal to the diarrhoea and malabsorption resulting in malnutrition , anaemia and other biochemical disturbances. A study therefore was undertaken to establish whether eradication of the organisms in HIV related diarrhoea with Albendazole leads to regeneration of the intestinal mucosa villi. If regeneration does occur it would have clinical significance as diarrhoea, malabsorption and consequently the malnutrition and biochemical disturbances should improve.

This study follows previous work that has already demonstrated in a double blind randomised placebo controlled trial that Albendazole in patients with persistent HIV - related diarrhoea and Karnofsky `s score between 50 and 70 got 50 % decrease in diarrhoea (Kelly et al , 1996).

AIMS

1. To study the current pattern and prevalence of parasites and bacteria in HIV - related diarrhoea

2. To establish the protozoal organisms in HIV - related diarrhoea that are susceptable to Albendazole thearpy

3. To observe the reponse of HIV related enteropathy to parasite eradication in HIV - related diarrhoea

SUBJECTS AND METHOD:

The study required ethical approval from the University of Zambia Research and Ethics Committee. Approval was obtained from the Committee on 31st May 1995 . The study was done from January to October 1996. 150 patients were consecutively recruited from the University Teaching Hospital medical wards , University Teaching Hospital outpatient clinic and three Lusaka Urban Health Centres (Chawama, Chipata and George). 54 of the patients were female and 100 were male. All patients gave written informed consent before being recruited to the study. Subjects were those with diarrhoea of a month or more in duration and with a Karnofsky's score of between 30 and 90 (see appendix). The purpose , nature of the study and the procedures done were fully explained to the patients at the time of recruitment . Once recruited, patients had three specimens of stools submitted on three consecutive days for bacteriological and parasitological studies. Using the modified Ziel Neelsen staining techinique the stool was examined for *Isospora belli* and *Cryptosporidium* and trichome stain for

Microsporidia. On an appointed day they came for upper gastrointestinal endoscopy and duodenal biopies were collected. Before endoscopy, blood was collected for HIV, full blood count , CD 4 and CD 8 counts. They had their weights and mid - arm circumference taken.

Eight duodenal biopsies were obtained . 2 biopsies were for histopathology for the purpose of determining the microscopic appearance of the mucosa.

After endoscopy and stool examination, patients were commenced on 400 mg twice daily dose of Albendazole for two weeks. They were also given oral rehydration salts and 30 mg three times a day of codeine phosphate. They were provided with a card for a daily record of the frequency and nature of diarrhoea. If any adverse effects occurred while taking the drugs they were to report immediately to the endoscopy clinic. At three weeks subjects came back for review. They had weight, mid - arm circumference and Karnofsky score determined. Three specimens of stool were again submitted on three consecutive days. If patients initially had Isospora belli isolated and at three weeks the organism still appeared in the stool they had Co trimoxazole 2 tabs 4 times a day for ten days supplied. The final review was after another three weeks, that is six weeks from the time of first endoscopy . At this visit they had again weights , mid arm circumference and Karnofsky score determined. Blood was collected for CD 4 and CD 8 counts. Repeat endoscopy was carried out for the collection of duodenal biopsies and evaluation of oesophageal and gastric abnormalities.

Morphometric analysis of the duodenal biopsies was done using computer aided microscopy. The technique is based on interactive

computer graphics and allows automatic measurements based on grey - level segmentation.The observer defines areas and lengths for measurement and does the analysis of the specimens in a rapid and efficient manner (Slavin G et al . 1980).

Analysis of results and statistical methods

Data were entered in the statistics database package ' Epi - info ` ,version 6.0 (WHO , Geneva). Analysis of mucosal response was based on two criteria :

a) Clinical response , defined as

0 no response

1 full response , with complete absence of diarrhoea at 6 weeks

2 partial response , in between these two extremes

b) Parasitological response , defined as elimination of a parasite identified at recruitment by 6 weeks .

RESULTS

83 patients completed the study of whom 25 were female and 59 were male but 70 were lost to follow up. Some of the lost died within the 6 weeks period of follow up , another group could not be traced either because they had moved houses or had supplied wrong addresses.

TABLES

AGE DISTRIBUTION OF PATIENTS

15 - 24 YEARS	25 - 34 YEARS	35 - 44 YEARS	45 - 54 YEARS	55 - 64 YEARS	65 - 74 YEARS
15	39	16	8	3	1

KARNOFSKY SCORES AT RECRUITMENT

KS SCORE	30	40	50	60	70	80	90
NUMBER OF PATIENTS	3	6	6	14	27	20	7

KS SCORE = KARNOFSKY SCORE

CD4 COUNTS DISTRIBUTION OF THE STUDY GROUP

< 50	27 %
50 - 100	49 %
> 200	24 %

ISOLATION OF ORGANISMS

PARASITES ISOLATED FROM INITIAL STOOL SAMPLES OF 83 PATIENTS

Parasites isolated	n	%
Isospora belli	34	40.9
Microsporidium	12	14.4
Cryptosporidium	3	3.6
Giardia	0	0
Strongyloides stercolaris	2	2.4
Blastocystis hominis	0	0
E. histolyticum	0	0
Yeast	17	20.4
Other	1	1.2

BACTERIA ISOLATED

NUMBER OF STOOL SAMPLES STUDIED	67
SALMONELLA	1
SHIGELLA	6

ORGANISMS ISOLATED AFTER ALBENDAZOLE THERAPY AT THIRD WEEK FOLLOW UP

Number of samples examined	Crypto	Iso	Micro	Strongy	Yeast	Others
78	0	27	6	0	14	2

Cyrpto = Cryptosporidium, Iso = Isospora belli, Micro = Microsporidium

Strongy = Strongyoides stercolaris

PARASITES ISOLATED AT SIXTH WEEK OF FOLLOW UP

Number of stool samples examined	Crypto	Iso	Micro
80	1	25	9

BACTERIA ISOLATED AT SIX WEEKS OF FOLLOW UP

Number of samples examined	Salmonella	Shigella
80	4	5

RESPONSE TO ALBENDAZOLE

CLINICAL RESPONSE

No response	Partial response	Complete response
21	29	33

MEAN CD4 ACCORDING TO CLINICAL RESPONSE

No response	112
Partial response	147
Complete response	194

PARASITE ERADICATION

CRYPTOSPORIDIUM

Cryptosporidium was eradicated in all three cases who completed the study by Albendazole.

MICROSPORIDIUM

BASELINE	AT 3 WEEKS		AT 6 WEEKS	
POSITIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
11	5	6	3	8

POSITIVE GROUP AT 3 WEEKS

NUMBER POSITIVE	POSITIVE AT 6 WEEKS	NEGATIVE AT 6 WEEKS
5	1	4

NEGATIVE GROUP AT 3 WEEKS

NUMBER NEGATIVE	POSITIVE AT 6 WEEKS	NEGATIVE AT 6 WEEKS
6	2	4

NEGATIVE GROUP FOR MICROSPORIDIUM AT BASELNE

BASELINE	AT 6 WEEKS	
NUMBER NEGATIVE	POSITIVE	NEGATIVE
67	5	62

ISOSPORA BELLI

NUMBER POSITIVE AT BASELINE	POSITIVE AT 3 WEEKS	NEGATIVE AT 3 WEEKS
34	27	7

10 DAYS Co trimoxazole THERAPY AT 3 WEEKS

CO TRIMOXAZOLE RECIPIENT AT 3 WEEKS	**POSITIVE AT 6 WEEKS AFTER 10 DAYS THERAPY**	**NEGATIVE AT 6 WEEKS AFTER 10 DAYS THERAPY**
18	13	5

MUCOSAL CHANGES WITH THERAPY

So far only 17 paired biopsies have been analysed. The examination of the rest of the biopsies is still going on.

PRESENCE OF PARASITES I N 17 BIOPSIES

POSITIVE FOR PARASITES	NEGATIVE FOR PARASITES
11	6

PARASITE ERADICATION IN 11 BIOPSIES

PARASITE ERADICATION	NO PARASITE ERADICATION
5	6

VILLOUS CHANGES AFTER TREATMENT

Mean difference in Villous height in the two groups of patients following treatment measured in micrometers

MEAN DIFFERENCE IN PATIENTS WITH PARASITES ERADICATED	MEAN DIFFERENCE IN PATIENTS STILL WITH PARASITE AFTER THERAPY
- 2.4 micrometers	9 micrometers

P value = 0.72

MUCOSAL RESPONSE AFTER THERAPY

NUMBER OF PATIENTS SHOWING MUCOSAL RESPONSE	NUMBER OF PATIENTS SHOWING NO MUCOSAL RESPONSE
8	9

Numbers include parasite negative biopsies at beginning of study

MEAN CD4s

IN PATIENTS WHOSE MUCOSA GOT BETTER	IN PATIENTS WHOSE MUCOSA NEVER GOT BETTER
194 cells/μl	154 cells/μl

p value = .4

MORTALITY

15 patients died during the 6 weeks of follow up

Median CD4

IN SURVIVORS AT 6 WEEKS	IN THOSE DEAD BY 6 WEEKS
135 cells/μl	91 cells/μl

P = 0.17

Mean body mass index

IN SURVIVORS AT 6 WEEKS	IN THOSE DEAD BY 6 WEEKS
17.8 kg/m^2	15.8 kg/m^2

P = 0.003 by ANOVA

MEAN VILLOUS HEIGHT

IN THOSE THAT SURVIVED	IN THOSE THAT DIED
365 μm	328 μm

MEAN CRYPT DEPTHS

IN THOSE THAT SURVIVED	IN THOSE THAT DIED
182 μm	158 μm

p = 0 .19

DISCUSSION

Age distribution

The greater number of the patients studied were between 25 and 34 years which is the economically active age group. This is the productive population group and illness such as this will seriously affect the socio - economic well being of the community and the nation.

Organisms isolated

The study shows that protozoal organisms are still the leading cause of diarrhoea in HIV infected patients in Lusaka as has been demonstrated by previous studies (Conlon et al. 1990, Kelly et al). In the present study, *Isospora belli* was the commonest organism isolated followed by Microsporidium. *Cryptosporidium* was the third commonest protozoal organism . Like the two previous studies *Isospora belli , Microsporidia* and *Cryptosporidium* remain the leading cause of HIV- related persistent diarrhoea. The difference in the parasite profile between this study and the previous study by Kelly et al is that *Isospora belli* is the most frequently isolated organism and *Microsporidium* is second where as in the previous study the reverse was the case. *Salmonella* and *Shigella* were the common bacterial organisms isolated although bacterial organisms were not the leading cause of diarrhoea in the group of patients studied. Bacterial organisms seem to be isolated less frequently in HIV patients with persistent diarrhoea in Zambia .

Albendazole therapy

Previous studies have shown that Albendazole leads to resolution of diarrhoea in 50 % of patients with a Karnofsky`s score between 50 and 80. In the present study Albendazole 800 mg twice a day for 2 weeks seems to eradicate *Microsoporidum* in 73% of the patients who completed the study. This finding is important considering that *Microsporidium* is one of the leading causes of persistent diarrhoea HIV - infected patients . After two weeks of Albendazole therapy 6 of the 11 initially positive samples were negative for *Microsporidium*. 5 samples still had the organisms despite the 2 weeks of therapy. At the end of 6 weeks 4 out of the 5 positive patients at 3 weeks had cleared the organism and only 1 remained positive. Of the 6 negative patients at 3 weeks 2 had *Microsporidium* at 6 weeks and 4 remained negative. Therefore a total of 8 patients were negative for the organism at 6 weeks. The two who had the infection could have been reinfected , relapsed or indeed failed diagnosis at 3 weeks. Weight appears to have an effect on ability of Albendazole to eradicate *Microsporidium*. The responders had a higher mean body mass index (17.5 kg /m ^{2}) than the non -responders (16.6 kg/m^2) . All patients except two with *Microsporidia* had Karnofsky `s score between 70 and 80. Of the two, one with a score of 50 responded and the other with a score of 40 did not respond. Since these patients had the same range of the score, this parameter did not affect clearance but it does at a lower score as shown in the earlier study by Kelly et al 1996 that patients with a score above 50 have better response than those with a lower score. CD4 count also affected clinical response. Patients with a higher CD4 did better than those with a lower score, the difference showing statistical significant ($p = 0.01$). Therefore patients with a higher body mass index , Karnofsky's score and CD4 count have better response to therapy.

Cryptosporidium

Cryptosporidium was isolated in three of the 83 samples of stool studied. Others were lost to follow up. All the three cases cleared the organisms after two weeks of Albendazole. If this clearance is due to the Albendazole this could prove very useful in the treatment of *Cryptosporidium* which currently does not have an established effective treatment except for the macrolide antibiotics , spiramycin which showed some early promise but have not stood the test of time (Farthing , 1993) or Zidovudine which is beyond reach of many in developing countries. However, the sample is very small for statistical significance.

Isospora belli

Isospora belli was the most commonly isolated organism in the group of patients studied. 13 cases (41 %) had *Isospora belli* eradicated after Albendazole therapy. However some could have been spontaneous remissions due to the placebo effect. 27 patients were still positive for *Isospora belli* at the third week visit showing no response to Albendazole. 18 of these were further put on Co - trimoxazole 960 mg 4 times a day for 10 days. Stools at six weeks showed negative results in 5 (28 %) cases and 13 (72 %) were still positive for the parasite. However 10 (55 %) of this group of patients experienced complete clinical response and 5 (28 %) had partial response. 3 (17 %) did not experience any response. There was not much age and Karnofsky` s score difference between both the clinical and parasitological responders and non responders. The group which had the parasite eradicated had a higher mean body mass index (17.8) as compared to the group with clinical response but no parasite eradication (16.6). It appeared also from the study that presence of other infections like tuberculosis affected response.

The low eradiaction rate of the parasite agrees with what has been stated that antimicrobial chemotherapy for intestinal coccidiosis cannot predictably eradicate infection (Farthing , 1993) . The findings however show that Co trimoxazole has some effect on Isospora belli as has been shown by other studies (Gazzard B et al 1993) .

Clinical response to Albendazole therapy

Of the 83 patients 33 had complete response and 29 partial response. Parasite eradication however was lower than clinical response. This could be possibly due to the fact that in some patients with the organisms but with clinical response, the organism could have been reduced to levels that does not cause disease hence the clinical response despite the presence of the organism.

Villous response to therapy

From the 17 paired biopsies studied it appears that eradication of intestinal parasites does not lead to regeneration of villi. In some patients the villi continued degenerating. There was no statistical difference in mean villous height difference between the group of patients that had parasites eradicated and the non responders (p = 0 .72). The lack of response in villous height is contrary to expectations that eradication of parasites should lead to regeneration of the villi. In other infection states eradication of parasites has lead to villi regeneration. De worming has been shown to lead to regeneration of the intestinal mucosal villi (Sheehy TW et al , 1962). Lindenman et al (1971) also demonstrated that mucosal villi of patients with enteropathy due to repeated parasitic

infections of the intestines improved after exposure to parasites ceased. It is not clear why the villi of the patients under the present study failed to improve.There are a number of possibilities that could have contributed to this failure .The first factor could have been the 6 weeks duration of follow up. The period could have been too short for the villi to regenerate. In Lindenman's study, mucosal villi regeneration occured after many months. In the present study repeat biopsies were done after 6 weeks for fear that if the period of follow up before biopsies was longer many of the patients would be lost . The other factor affecting villi regeneration could have been the patient's nutritional status. Many of the patients were malnourished and this nutritional state could have affected the ability of mucosal regeneration. The HIV virus itself could have contributed to the inability of the villi regeneration as it has been postulated that it can contribute to the mucosal abnormalities.

Factors associated with mortality

CD 4 count comparisons were made between the survivors at 6 weeks and those that died. The count was higher in survivors but the difference was not statistically significant (p = 0.71 by Kruskal - Wallis test) . Body mass index was found to be higher in the survivors and the difference was statistically significant (p = 0.003 by ANOVA). Nutritional status in this study was a predictor of death. Villous height was greater in the patients that died and was not a good indicator of ill health. Finding of bacterial or parasitic infection was not associated with death.

CONCLUSION

Isospora belli, Microsporidium and *Cryptosporidium* are still the leading organisms causing persistent diarrhoea in HIV infected patients. 83 patients completed 6 weeks of follow up. 40 % of these patients completely responded to Albendazole therapy and 35 % had partial response. Only 25 % failed to show any response. Co - trimoxazole did not eradicate *Isospora belli* in 13 out of 18 cases but more patients on the drug had clinical response. Patients with higher body mass index and CD4 counts have better clinical response than those with lower readings. Villi did not regenerate in the patients who had parasites eradicated. Survivors had a better nutritional status than those who died within the 6 weeks period of follow up. From the study it has been shown that Albendazole is beneficial to patients with persistent diarrhoea. It eradicates *Microsporidium* in 73 % of and *Isospora belli* in 41% of cases but eradication did not lead to villi regeneration.

RECOMMENDATIONS

This study supports other studies which have shown that Albendazole and Co trimoxazole are useful in treatment of HIV - related persistent diarrhoea. Use of the above 2 drugs would prove helpful among physiscians in treating diarrhoea in HIV infected patients especially when the responsible organisms which respond to the two drugs have been isolated. More work needs to be done to find an affordable agent effective against *Cryptosporidium.*

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KARNOFSKY SCORE CHART

Able to carry on normal activity; no special care needed:

Score	Status
100	Normal: no complaint, no evidence of disease.
90	Able to carry on normal activity; minor signs of disease
80	Normal activity with effort; some signs or symptoms of disease

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Unable to work; able to live at home and care for most personal needs; a varying amount of assistance needed:

Score	Status
70	Cares for self; unable to carry on normal activity or do active work
60	Requires occasional assistance and frequent medical care
50	Requires considerable assistance and frequent medical care

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Unable to work; able to live at home and care for most personal needs; a varying amount of assistance needed:

Score	Status
40	Disabled; requires special care and assistance
30	Severely disabled; hospitalisation necessary and active support treatment is necessary
20	Very sick; hospitalisation necessary and active supportive treatment is necessary
10	Moribund; fatal process progressing rapidly
00	Dead