

Relationship Between Digestive Tract Pathogens and HIV Infection in Onitsha Metropolis

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Abstract: Four hundred and seventy eight (478) individuals who exhibited some manifestation of chronic and debilitating illness including persistent cough, skin cancer and dermatitis, multiple lymph adenitis, diarrhea and enteritis, genital sore, urethritis, vaginitis and weight loss, were examined to establish relationships between human immunodeficiency virus infection (HIV) and digestive tract pathogens in Onitsha metropolis. There was a significant relationship between HIV positive individuals and digestive tract diseases with HIV positives recording more digestive tract diseases.

Key words: Digestive pathogens • HIV infection • Onitsha

INTRODUCTION

The digestive tract is one of the most common sites for clinical expression of human immune-deficiency virus infection [1]. Many studies show that victims express oral Candidiasis, approximately one third of patients develop peri-rectal lesions due to *Herpes simplex virus* [2], 30%-80% experience chronic or intermittent diarrhea. Several gastro intestinal diseases appear to be prevalent in these patients including visceral Kaposi sarcoma, Microsporidiosis, Cryptosporidiasis, Cytomegalovirus of the digestive tract [3, 4]. Many HIV infected patients show symptoms of persistent diarrhea, fever and gastritis [5].

The cause of gastroenteritis may be due to proliferation of intestinal opportunistic parasites e.g. protozoa, especially *Salmonella* species, fungi and viruses resulting from lowered immunity [6]. Persistent gastroenteritis with fever and other symptoms of AIDS including weight loss and dermatological manifestations should raise a warning signal to clinicians and other health workers. It is possible that persistent digestive tract disease may be indicative of HIV infection but the exact relationship between the two is not clear especially with regard to our Nigerian environment which may present different types of intestinal parasites from such

types seen in caucatain HIV individuals. It was therefore necessary to evaluate intestinal parasites in HIV positive and negative individuals to determine the extent and distribution of the parasites.

MATERIALS AND METHODS

Sampled Population: The study examined four hundred and seventy eight (478) individuals. Among these were fifty seven (57) individuals that showed symptoms of digestive tract infection, including diarrhea and stomach.

Collection of Samples: About 1-2 grams of freshly passed stool samples were collected from each patient in a universal specimen container and labeled. Serum samples obtained from coagulated blood samples were used for HIV and Western blot analyses. Using Savyon Diagnostic Ashdod Israel and Western blot for confirmatory test by Biorad Novo blot from Paris France respectively.

Stool Routine Examination: The stool samples were examined macroscopically for appearance prevalence of blood mucus and texture. A simple normal saline mount was made by picking a little quantity of the stool taken from suspicious sites and mounted in normal saline on a clean glass slide.

Stool Concentration Technique: All stool samples that yielded no cysts larva, ova or protozoa in the simple normal saline direct wet film examination were subjected to concentration technique as follows:

About 1-2grams of stool were placed in a 20ml glass tubes and sieved into small beakers using guaze strainers. This was transferred into 10ml glass tubes i.e. 6ml of suspension and 3ml of ether added, mixed well and centrifuged at 3000 rotation per minute for 1 minute.

By means of clean Pasteur pipettes, a few drops of the deposits were taken and mounted on slides, covered with cover slip and examined under low and high power objective lens of the microscope [7].

Isolation of Digestive Tract Pathogens from Stool

Bacteria: Portions of the stool were picked by means of sterile wire loop previously flamed over Bunsen burner and inoculated on Xylose lysine dextrose (XLD) agar. The inocula were streaked out on the plates using the wire loop flamed in-between streaks to avoid crowded growth in the XLD agar. The plates were incubated at 37°C over night.

Fungus: Using a flamed wire loop, small portions of the stool samples were picked up and inoculated into Sabouraud agar plates, slants and slides in pairs and incubated for 14 days at 37°C also observing daily for growths.

Identification of Digestive Tract Bacterial

Pathogens: Yellow colonies growing on XLD agar with black centers were suspected Salmonella colonies. These suspected colonies were sub-cultured on Nutrient agar to stabilize the species and the pure growths subjected to biochemical identification as well as motility test by hanging drop method.

Biochemical Identification of Digestive Tract Pathogens:

Sterile wire were used to pick the suspected colonies and introduced into Durham fermentation tubes of glucose, lactose, sucrose and manitol. Also, the wire stabs of suspected colonies were pierced through Triple sugar iron agar (T.S.I.A) and sub-cultured onto Mackonkey agar to ensure purity. The fermentation tubes, TSIA tubes and plates were incubated at 37°C over night. All colonies of suspected bacteria that were motile, fermented, TSIA slant with alkaline (pink) and acid with gas but were identified as *Salmonella typhimurium*. The fermentation tubes with motile organisms that fermented glucose, did not ferment

lactose and sucrose, acid manitol and orange yellow acid (only) butt of TSIA were identified as *Salmonella typhi*.

Serological Identification of Digestive Tract Bacterial

Isolates: A drop of saline was applied on a clean glass slide and the bacteria colonies under test on Nutrient agar plates were collected with a sterile wire loop and emulsified on the slide to form moderate suspensions. Monovalent Salmonella type specific antisera (Oxford England) were added and steered slightly and observed for characteristic agglutination reaction.

Identification of Digestive Tract Fungal Isolates:

The Sabouraud agar slants and plates that grew suspected creamy colonies were tested as follows. The colonies were tested as follows: The colonies were picked up with the tip of sterile capillary pipette and gently transferred and emulsified in 0.5ml sterile serum in a small test tube. Pooled human sera were used. The above was positive repeated in a second tube using a known *Candida albicans* culture as positive control. The tubes were incubated at 37°C for 2^{1/2} hours. The sera were mixed and transferred by means of a pipette to a slide covered with coverslip. The slides were examined under high power and low power of the microscope for the presence of short, lateral hyphal filaments (Germ tube) formed by the yeast cells [8].

Identification of Digestive Tract Fungal Parasites:

The X10 and X40 objectives of the microscopes were used to examine the Slides that were mounted with emulsified stools and parasites identified pictorially continuously changing roundish structures with more than four nuclei were identified as *Entamoeba histolytica*.

Roundish and occasionally oblong/oval flagellated highly motile cells were identified as *Trichomonas hominis*. By means of Pasteur pipette, drops of Lugol's iodine were applied to the slides to highlight and confirm the nuclear of *Entamoeba histolytica* [9].

Antibiotic Sensitivity Test:

Each of the isolates *S. typhimurium* and *S. typhi* were subjected to antibiotic sensitivity as follows: relevant antibiotic mentioned discs e.g. Gentamycin, Amoxycillin, Perflacin, Chloramphenicol, Ampicillin, Tetracyclin, Ofloclin, Ciproxin. Sporidex Ciproval and Ceporex were placed on each Mueller-Hinton sensitivity solid agar plates that has been previously seeded evenly with the test organisms diluted to 1 x 10^{4.5} organisms/ml, disc diffusion method and incubated over night at 37°C [10].

Table 1: Relationship Between Digestive Tract Diseases (DTD) and HIV Infection in Onitsha Metropolis

	HIV positive	Number affected.	HIV Negative		Relative Risk	Chi. Sq	Sig. at P=0.05
	Number Affected	Number of positive	Number Affected	Total			
Digestive Tract Diseases	34	20.12	23	57	2.26	1.9	Sig.
No of Digestive Tract Disease	135		286	421			
Total	169		309	478			

After incubation, the zones of inhibition surrounding the antibiotics sensitivity discs were taken as a measure as a measure of the inhibitory and bactericidal power of the drug against the particular test organisms and denoted as sensitive(s). The disc having the test organisms still growing around the disc were adjudged resistant.

There was significant relationship between digestive tract infection and HIV ($X^2 = 16.7$, $df = 1$, $P = 0.05$, $N = 478$). When total pathogens isolated was taken into consideration. There was no significant difference between occurrence of DTD in HIV positives and negatives statistically although there was high relative risk of those with DTD having HIV infection.

RESULTS

Relationship Between Digestive Tract Diseases and HIV Infection in Onitsha: Individuals under test who had intestinal protozoal, bacterial, or fungal organism and HIV infection were thirty-four (34) in number and those without HIV infection were twenty three (23). The prevalence rates of those affected by the parasitic infections among the HIV positives and negatives were 20.12% and 7.44% respectively.

Those with no digestive tract infection but were HIV positive were one hundred and thirty five (135) and HIV negatives were two hundred and eighty six (286) in number. The relative risk was 2.26. There was a significant relationship between HIV positive individuals and digestive tract diseases with HIV positives recording more digestive tract diseases ($X^2 = 16.7$, $df = 1$ and level of significance $P = 0.05$).

DISCUSSION

One of the earliest recorded symptoms of HIV infection was persistent gastro enteritis, fever and diarrhea [11, 12 and 13]. The chance by those with HIV infection (Relative risk 1.9)

The immune-compromised situation created by HIV infection could have resulted in the proliferation of these parasites and their toxins thus giving rise to digestive tract diseases.

Contrary to our local environment especially Onitsha metropolis and perhaps Anambra State, the commonest pathogens identified in stools of HIV sufferers among Caucasians particularly in USA included *Pneumocystis carinii* and *Cytomegalo virus* of the digestive tract and *Cypstosporidium Carinii* [11].

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