American-Eurasian Journal of Scientific Research 10 (3): 182-185, 2015
ISSN 1818-6785

DOI: 10.5829/idosi.aejsr.2015.10.3.1151

Relationship Between Digestive Tract Pathogens and HIV in Onitsha Metropolis with Respect to Gender

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Abstract: Four hundred and seventy eight 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 immuno-deficiency 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 with females recording higher prevalence rate of 21.08%.

Key words: HIV · Digestive tract diseases · Diarrhea and enteritis

INTRODUCTION

The digestive tract is one of the most common sites for clinical expression of human immune-deficiency virus infection [1, 2]. Many studies show that victims express oral candidiasis, approximately one third of patients develop peri-rectal lesions due to herpes simplex virus [3], 30%-80% experience chronic or intermittent diarrhea. Several gastro intestinal diseases appear to be prevalent in these patients including visceral Kaposis sarcoma. Microsporidiosis, Crytosporidiasis, Cytomegalovirus of the digestive tract [4, 5]. Many HIV infected patients show symptoms of persistent diarrhea, fever and gastritis [6].

The cause of gastroenteritis may be due to proliferation of intestinal opportunistic parasites e.g protozoa, bacteria especially Salmonella species, fungi and viruses resulting from lowered immunity [7-13]. 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 caucatian 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 with respect to gender.

MATERIALS AND METHODS

Sample Population: The study examined four hundred and seventy eight (478) individuals.

Collection of Samples: All information such as gender was obtained and data recorded. About 1-2grams 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 by ELISA technic using ICT from Savyon Diagnostics using a kit by Biorad Paris France respectively.

Stool Routine Examination: The stool samples were examined macroscopically for 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 a 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:

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About 1-2grams of stool were placed in a 2oml 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 pasture 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 (Ramnik, 1990) [9].

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 agar. The inocula were streaked out on the plates using the wire loop flamed in-between streaks to avoid crowded growths 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 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 stabs 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 whoever was 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 glucose and manitol with acid and gas, lactose and sucrose not fermented, TSIA slant with alkaline (pink) and acid with gas butt 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 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 Sabourated agar slants and plates that grew suspected creamy 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 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 pipette to a slide covered with coverslip. The slides were examined under high power and low powers of the microscope for the presence of short, lateral hyphal filaments (Green tube) formed by the yeast cells [9].

Identification of Digestive Tract 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 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 Enta-amoeba 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, Oflocin, 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 $1x10^{4.5}$ organisms/ml, disc diffusion method and incubated over night at 37°C [11].

After incubation, the zones of inhibition surrounding the antibiotic sensitivity discs were taken as a measure of the inhibitory and bactericidal power of the drug against

Table 1: Distribution of Digestive Tract Diseases in Onitsha Metropolis and Their Relationship with Hiv Infection with Respect to Gender

	HIV Positive				HIV Negative					
	M	F	Total isolates	Total HIV Positive	M	F	Total isolates	Total HIV Negative	Total HIV Positive and Negative isolates	Total HIV Positive and Negative
Trichomonas hominis	4.66	5.79	25		1.27	2.07	8		33	
Enta-Amoeba histolytica	2.54	6.20	21		1.69	2.89	11		32	
Candida albicans	3.81	7.02	26		2.54	4.13	16		42	
Salmonella typhi	1.69	0.82	6		-	0.83	2		8	
Salmonella typhimurium	0.42	1.24	4		0.42	0.41	2		6	
Total	13.12	21.08	82	169			39	309	121	478
Sig. difference at P ≤0.05 for DTP in HIV Positive and Negative in Gender	Sig. X^2=7.54	Sig. X^2=10.6								

There was significant difference in occurrence of DTD in HIV positives and negatives.

the particular test organisms and denoted as sensitive(s). The discs having the test organisms still growing around the discs were adjudged resistant.

Statistical comparison between digestive tract pathogens in HIV positive and negative individuals were carried out and results noted.

RESULTS

HIV Positive: Thirty-four cases of digestive tract diseases yielded eighty two (82) isolates distributed as follows: Females recorded higher prevalence rate of *Trichomonas hominis* than males 5.79% and 4.66% respectively. Total was twenty-five (25).

Females again recorded higher prevalence of *Enta-amoeba histolytica* than males 6.20% and 2.54%. Total = twenty-one (21).

Females again recorded higher prevalence rate of *Candida albicans* 7.02% and 3.81% respectively.

Males recorded higher prevalence rate of *Salmonella typhi* than females 1.69% and 0.83%. Total was six (6).

Females recorded higher prevalence rate of *Salmonella typhimurium* than males 1.24% and 0.42%

Females recorded higher prevalence than males for total pathogens isolated 21.08% and 13.12% respectively for HIV positives.

HIV Negative: The twenty-three (23) cases of digestive tract diseases yielded thirty-nine isolates distributed as seen in Table 1 below:

There was significant difference of DTP in HIV positive and negative for males and females with more DTP in HIV positive for both males and females.

However, males and females that were HIV positive showed a statistical difference at P≤0.05 with more females having more DTP X^2 5.71.

Males and females who were HIV negative showed no statistical difference (X^2 3.07).

DISCUSSION

Gender distribution of each of the digestive tract pathogens displayed no specific pattern and thus could not be considered gender specific as shown in table 1.

There was significant difference in occurrence of Digestive tract disease in HIV positive and negative with more DTP in females with HIV positive.

There was a significant difference between males and females with HIV positive having more total DTP in females than males. However for males and females with HIV negative there was no significant difference in occurrence of DTP.

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